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# Drought induced responses in hybrid and balsam poplars

by

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*To my mother for guidance, love and support*

*To my grandma Graciela for her lessons for life*

*To my sister and nephews for keeping their hope*

*To Roger for supporting my dreams*

## Abstract

Six hybrid poplar clones and balsam poplar (*Populus balsamifera* L.) were subjected to different water availability regimes to determine differences in their drought resistance mechanisms and recovery capacity. Plants were subjected to mild drought stress ($\Psi_{soil}$ -0.05 MPa), severe drought stress ($\Psi_{soil}$ -2.0 MPa), and drought recovery following re-watering at 30 minutes, one day and (or) two days ($\Psi_{soil}$ -0.004 MPa). A functional coordination by a timed response of the stomatal aperture to reduced stem hydraulic conductivity was likely the main factor associated with preventing extensive loss of vessel conductivity due to induced cavitation in these poplars. The studied poplars reached a relatively high percentage of loss of conductivity at lower xylem water potential values compared with other tree species. It was noted that, although stomatal regulation was involved in preventing xylem cavitation, xylem architecture also played an important role in resistance to xylem cavitation. In addition, most of the poplars displayed high cell wall elasticity, maintaining cell turgor pressure at lower tissue water contents. Accumulation of carbohydrates and proline were not a significant factor in drought resistance, and varied between the clones and tissues. Root hydraulic conductivity also differed between treatments and poplar clones. Increases in conductivity were observed after the re-watering treatment pointing to the importance of root water transport during drought recovery. In general, combined drought-resistant mechanisms and a recovery capacity were found in these poplar clones: Northwest showed lower vulnerability to xylem cavitation, lower cell wall elasticity and developed lower leaf water potential to maintain a positive water potential gradient for water uptake from drying soil. Walker and

balsam poplar displayed a drought tolerance strategy maintaining open stomatas, relatively high root hydraulic conductivity and moderate resistance to xylem cavitation. The Berlin clone was relatively sensitive to cavitation and had poor stomatal control suggesting that the plants of this clone may be susceptible to drought stress conditions. Assiniboine was more sensitive to xylem embolism at relatively high stem water potentials. Finally, Okanese and P38P38 showed an avoidance strategy characterized by stomatal closure to maintain high $\Psi_{stem}$ values since their xylem was found to be relatively vulnerable to cavitation.

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# Chapter One
# Introduction

Recent climate change is associated with an increase in global temperatures. These changes in temperature affect the hydrological cycle by increasing evapotranspiration which modifies the extent and distribution of precipitation (IPCC 2007). As a result of climate change, the length of drought periods is expected to increase as global temperatures continue to increase (Hansen *et al.*, 1999; Saxe *et al.*, 2001). In the Prairie Provinces of Western Canada, drought conditions often occur in the summer and high precipitation, in the form of snowfall is common during the winter months (Zhang *et al.*, 2000; 2001; Schindler & Donahue, 2006). However, due to increasing temperatures, more intense periods of drought are expected since the increases in precipitation in the Canadian Prairies will not be enough to balance the increase in evapotranspiration (Easterling *et al.*, 2000). These changes may affect vegetation distribution and productivity, and alter regional carbon and nutrient cycles (IPCC 2007).

*Populus* is an ecologically and commercially important genus for pulp production in Canada. Biomass production in poplars is limited by water availability and environmental conditions (Hinckley *et al.*, 1994). As 35% of the world's natural poplars (28.3 million hectares) are located in Canada (FAO, 2004), it is important to understand the characteristics and mechanisms of drought resistance in this genus.

In general, drought stress affects plant phenology, growth, carbon assimilation and reproduction processes (Bacelar *et al.*, 2007). Since the movement of water follows the water potential gradient from the soil to the atmosphere, decreases in soil water availability and increases in evaporative demand, lead to changes in plant water potential. In order to cope with decreased water potential, internal adjustments withing the plant are required (Polle *et al.*, 2006). These adjustments include physiological and metabolic changes resulting, in some cases, in morphological modifications. Some of these are temporary changes related to stomatal regulation, plant structural

modification, and changes in tissue osmotic potential and hydraulic conductances (Passioura, 1996; Monneveux & Belhassen 1996). Poplars are grown as intensively managed woody crop throughout many regions of the world, from dry to wet climate regions, and exhibit large genetic variations (Stettler *et al.*, 1996; Tuskan, 1998, Dickmann, 2001, Zhang *et al.*, 2004).

In northeastern Alberta, Alberta-Pacific Forest Industries Inc. (Al-Pac) has an extensive poplar-farming program with research focusing on selecting hybrids with improved drought-resistance characteristics. These poplar farm plantations are projected to supply approximately 12% of Al-Pac's fiber needs. In light of the importance of drought resistance for poplar growth in natural areas and for poplar farming, my study was designed to gain a better understanding of the drought physiology of the current operational clones and to assess the role of drought resistance mechanisms in hybrids and balsam poplars from Sections *Aigerios* Duby and *Tacamahaca* Spach (*Populus* spp). In addition, my research results will provide a basis for the establishment of screening techniques that are necessary to identify traits related to drought resistance, and to select poplar clones that are suitable for different site moisture conditions as new clones become available for use.

In this study, I hypothesized that the physiological features that could be used for the development of drought resistance tests include plant hydraulic conductance, vulnerability of xylem to cavitation, changes in cell-water relations, and osmoregulation. I also hypothesized that sensitivity of stomata to drought, high resistance of xylem to cavitation and high capacity for osmotic adjustment are among the most important characteristics responsible for differences in drought resistance between different poplars. The aim of this study was to investigate different drought resistance strategies in poplar clones by determining: (1) stomatal sensitivity to drought, (2) resistance of xylem to cavitation, (3) leaf cell wall elasticity, (4) accumulation of compatible solutes, (5) root hydraulic conductance, and (6) recovery capacity to xylem cavitation.

The present study was designed to understand and characterize the mechanisms of drought resistance in the following poplar clones: P38P38 (*P. balsamifera* x *P. simonii* cv. P38P38), Walker (*P. deltoides* x (*P.* x *petrowskyana*) cv. Walker), Okanese (Walker x *P. x petrowskyana* cv. Okanese), Northwest [P. balsamifera x P. deltoides cv. Northwest (Jackii hybrid)], Assiniboine (open-

pollinated Walker cv. Assiniboine), Berlin (*P. x berolinensis* cv. Berlin), and *P. balsamifera* L. Well-watered plants were compared with two levels of drought stress and two periods of rewatering (recovery treatments). Drought experiments were conducted in a greenhouse to facilitate precise control of environmental conditions.

Following the Introduction (Chapter 1), this thesis is divided into five chapters as follows. Chapter 2 is a literature review of the plant water relations and biology of poplars. The topics focus on soil water uptake, water transport and drought resistance mechanisms in plants; as well as the description and biology of the studied poplar clones. Chapter 3 describes the study exploring the mechanisms used by these poplars to deal with different water deficit levels and for the recovery from drought stress. Relationships were examined using vulnerability to cavitation measurements, stomatal conductance, leaf aperture and density, and water potential in leaves and stems. Stem xylem cavitation resistance was examined in well-watered plants with the Cavitron technique after inducing increases in xylem tension.

In Chapter 4, I studied turgor capacity and accumulation of organic solutes in leaves and roots in response to drought. I also investigated the relationship between solute accumulation and drought recovery capacity. I sought to gain a better understanding of osmotic adjustment processes by analyzing carbohydrate and free total amino acid content in leaves and roots. Pressure volume analyses were also carried out to examine various leaf water relationship parameters including cell elasticity, osmotic potential at full hydration and turgor loss point, and symplastic water fraction of the leaves. In Chapter 5, I examined transpiration, root hydraulic conductance, and stem xylem cavitation and subsequent recovery in drought-induced poplar clones. Finally, Chapter 6 synthesizes the results described in Chapters 2-5 along with general recommendations for suitable methods to measure drought resistance in poplars and future research needs.

## References


Bacelar EA, Moutinho-Pereira JM, Gonçalves BC, Ferreira HF, Correia CM (2007) Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. *Environ Exp Bot.* **60**, 183-192.

Dickmann DI (2001) An overview of the genus Populus. *In:* Poplar culture in North America. Part A, Chapter 1. Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J (Eds). NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada. Pp. 1-42.

Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate extremes: observations, modeling, and impacts. *Science* **289**, 2068-2074.

Hansen J, Ruedy R, Glascoe J, Sato M (1999) GISS analysis of surface temperature change. *Journal of Geophysical Research* **104**, 30997-31022.

Hinckley TM, Brooks JR, Cermak J, Ceulemans R, Kucera J, Meinzer FC, Roberts DA (1994) Water flux in a hybrid poplar stand. *Tree Physiol.* **14**, 1005-1018.

IPCC (2007) Climate Change 2007: The Physical Science Basis. Summary for Policymakers Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

Monneveux P, Belhassen E (1996) The diversity of drought adaptation in the wide. *Plant Growth Regul* **20**, 85-92.

Passioura JB (1996) Drought and drought tolerance. *Plant Growth Regul* **20**, 79-83.

Polle A, Altman A, Jiang X (2006) Towards genetic engineering for drought tolerance in trees. *In*: Tree Transgenesis. Fladung M. & Ewald D. (Eds). Springer-Verlag Berlin Heidelberg. Pp. 275-297.

Saxe H, Cannell MGR, Johnsen Ø, Ryan MG, Vourlitis G (2001) Tree and forest functioning in response to global warming. *New Phytol* **149**, 369–400.

Schindler DW, Donahue WF (2006) An impending water crisis in Canada's western prairie provinces. *PNAS* **103**, 7210-7216.

Stettler RF, Bradshaw HD, Jr Heilman PE, Hinckley TM (1996) Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa. 539 pp.

Tuskan GA (1998) Short-rotation woody crop supply systems in the United States: what do we know and what do we need to know? *Biomass Bioenergy* **14**, 307–315.

Zhang X, Vincent LA, Hogg WD, Niitsoo A (2000) Temperature and precipitation trends in Canada during the 20th century. *Atmos Ocean* **38**, 395-429.

Zhang X, Harvey KD, Hogg WD, Yuzyk TR. (2001) Trends in Canadian Streamflow. *Water Resour Res* **37**, 987–998.

Zhang X, Zang R, Li C (2004) Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. *Plant Sci* **166**, 791-797.

# Chapter Two

# Literature Review

## *2.1 Plant and soil water relations*

The Cohesion-Tension theory first proposed by Dixon & Joly (1894) is the widely accepted mechanism for the ascent of sap (Milburn, 1996; Cochard *et al.*, 2000; Steudle, 2001; Tyree, 2003; Angeles *et al.*, 2004). The driving force for water movement is generated by leaf transpirational water loss, transmitting tension through the continuous water column from leaves to roots (Van den Honert, 1948; Meinzer *et al.*, 2001). This water continuum from soil through the stem to leaf cells is known as the soil plant atmosphere continuum (SPAC). There are several factors involved in the process of water movement to roots; consequently the maintenance of this hydraulic system is essential to ensure a continuous water supply to leaves (Maseda & Fernández, 2006).

The amount of water in the soil that is available for plants corresponds to the water content of the soil and how that water is held by largely matric forces. Its availability depends primarily on the water potential at the root-soil interface and the hydraulic conductivity of the soil. The water potential at root-soil interface is controlled by the relative rates of flow to and removal by roots (Steudle, 2000a; Schulze *et al.*, 2005). The root system of growing plants is continually exploring more soil volume and absorption is more efficient in the zone behind the apex of root tips, where root hairs develop and the root cortex is not yet suberised. Hence, the soil mass occupied by a root system often consists of several zones of different water content (Kramer, 1969; Steudle, 2000b).

Water in the soil-plant-atmosphere continuum is affected by four main field forces: matric, gravitational, osmotic and water pressure. The sum of these forces is the water potential and is usually expressed in pressure units (energy/area) in megapascals (MPa) (Kirkham, 2005). In plants and soils, matric potential is a result of the adsorptive forces binding water molecules to the surface of soil particles, cell walls or macromolecules (adhesive forces). Gravimetric potential will depend on the height of the water above ground (i.e. the higher the water column, the greater the influence of gravity), osmotic potential is

a result of solute concentration in the cell or soil particles; and the pressure potential is a result of the intracellular hydrostatic pressure (turgor pressure) (Kramer & Boyer, 1995). In soils, matric forces dominate except in alkaline and saline soils where osmotic potential may be also important. Accordingly, the components for water potentials are expressed as follows:

$$\Psi_w = \Psi_p - \Psi_s + \Psi_g - \Psi_m \quad \text{(MPa)} \qquad [1]$$

where $\Psi_w$ is the total water potential, $\Psi_p$ is the pressure potential (or turgor potential in plants), $\Psi_s$ is the osmotic potential, $\Psi_g$ is the gravimetric potential, and $\Psi_m$ is the matric potential (Nilsen & Orcutt, 1996). In plants, except in tall trees where $\Psi_g$ needs to be considered, gravimetric potential and matric potentials are a small portion of the total water potential. Gravimetric potential increases 0.1 MPa for each 10 m increase in height; and normally leaf water potential ranges from -0.5 to -2.5 MPa. On the other hand, matric potential is not significant for cells at high tissue hydration; although in dehydrated tissues, or with large quantities of mucilage or colloids, matric potential becomes significant. If $\Psi_g$ and $\Psi_m$ are ignored, the relation of $\Psi_s$ and $\Psi_p$ to water content or cell volume may be explained in the Höfler-type diagram (see Baker, 1984; Kirkham, 2005). The Höfler diagram represents the changes in osmotic potential and cell water potential as the volume and the pressure potential change (Kramer, 1969). Thus, in a cell at zero turgor, water potential is equal to the osmotic potential, since pressure potential equals zero ($\Psi = \Psi_s$ and $\Psi_p = 0$). Water potential at full cell turgor equals zero, because pressure potential is the same as osmotic potential ($\Psi = 0$ and $\Psi_p = -\Psi_s$).

Maintenance of cell water status under drought, through cell wall elasticity and increased apoplastic water fraction, plays a significant role in plant stress response (Turner & Jones, 1980; Blake *et al.*, 1991). Elasticity determines how cell turgor changes with changes in leaf water content, and is quantified by the elastic modulus ($\varepsilon$) of the cell wall. Low values of $\varepsilon$ indicate more elastic cell walls and therefore greater ability to maintain positive turgor potential at relatively low cell water content. Conversely, a large $\varepsilon$ value results in a relatively rapid decrease in turgor pressure as drought stress increases, and this is associated

with a rapid decrease in water potential. An increase in elasticity stabilizes tissue water content instead of stabilizing turgor pressure (Nilsen & Orcutt, 1996).

### *2.2 Measurement of plant water relations*

The Scholander pressure chamber (Scholander *et al.*, 1965) was used in the present study to measure leaf and stem water potentials and to examine the relationship between leaf water content and water potential (pressure-volume method). The pressure chamber technique is widely used to analyze water potentials in plants in the greenhouse and the laboratory because it is quick and simple to use. This technique allows indirect estimates of the xylem pressure based on the assumption that tension in the xylem stream is equilibrated with the water potential of the leaf cells (Richie & Hinckley, 1979; Turner, 1988; Nilsen & Orcutt, 1996), and that water movement in the plant follows the principle of the cohesion-tension theory (Cochard *et al.*, 2001; Angeles *et al.*, 2004). According to Gardner & Rawlins (1965), as air pressure is applied to a sample in a chamber, the free energy of the water is raised. Increases in the applied pressure will increase the free energy of water by approximately $V\Delta P$, where V is the volume of the water in the sample, and $\Delta P$ is the pressure increase necessary to establish equilibrium between water in the system and outside.

The pressure volume (P-V) technique (Tyree & Hammel, 1972) allows for estimates of cell water relations under drought (Redfield & Zwiazek, 2002; Saito & Terashima, 2004; Lemcoff *et al.*, 2006; Castro-Díez & Navarro, 2007). A pressure volume curve describes the relationship between the tissue water content and the components of tissue water potential (Kirkham, 2005) as described in Chapter 4.

### *2.3 Root water uptake*

Absorption of water from soils is dominated by two mechanisms, active or osmotic absorption in slowly transpiring plants, and passive absorption in rapidly transpiring plants. In osmotic absorption the roots act as osmometers, while passive transport occurs by evaporation of water from leaf cells, decreasing water potential of the plant and inducing movement of water from the soil

(Kramer & Boyer, 1995). In roots, the transport pathway consists of radial transport across the epidermis, cortex and stele, and with axial transport, through the tracheary xylem elements (Steudle, 2000b). In the radial transport, there are three pathways for water movement from the epidermis across the cortex to the xylem: (1) apoplastic way through cell walls, (2) symplastic pathway by cells plasmodesmata, and (3) from cell to cell across cell walls, plasmalemmas, vacuoles, and tonoplasts; or by a combination of these three pathways (Kramer & Boyer, 1995). The cell to cell pathway is dominated by aquaporins or water channels, which are largely regulated by different internal or external parameters such as drought, salinity, temperature, nutrient status or diurnal rhythm (Steudle & Peterson 1998; Steudle, 2000b; Steudle & Ranahunge, 2007). In the axial transport, water moves by a differential pressure between roots and leaves through the non-living xylem vessels. A negative pressure is transmitted within the xylem to the roots.

Water flow through roots can be quantified as the root hydraulic conductance or root hydraulic conductivity (Van den Honert, 1948; Sperry *et al.*, 2002). Root hydraulic conductance is the ratio of the water flow rate and the difference in pressure causing the flow; while root hydraulic conductivity refers to the ratio of water flow rate and the pressure gradient in a specific segment length (Tyree & Ewers 1991; Tyree, 2003). Xylem transport from roots to leaves is determined by its hydraulic conductivity, and is proportional to the fourth power of vessel radius, as given by the Hagen-Poiseulle law (Zimmermann, 1983); where the water flow is proportional to the radius of the inner lumen of the vessel and to the pressure gradient (Zimmermann, 1983; Nilsen & Orcutt, 1996). Hydraulic conductance may fluctuate in response to external or internal factors such as xylem embolism, root anatomy, drought stress and salinity among others (Steudle, 2000b; Siemens & Zwiazek, 2004; Aroca *et al.*, 2005). Under abiotic stress, water potential becomes more negative in the soil and roots, reducing hydraulic conductivity from high values at saturation to near zero.

### *2.4 Xylem structure*

Secondary xylem in poplar consists of vessels, fibers, axial parenchyma, and ray parenchyma. Poplar wood is diffuse-porous, where the vessels are relatively uniform in size within a growth ring and there is no clearly defined earlywood and latewood. The vessels are relatively small and distributed radially (Zimmermann, 1978; Wiedenhoeft & Miller, 2005). They are connected by pit membranes for lateral water movement between vessels. These pits are organized in a honey comb fashion, for more efficient water passage. The pit borders provide mechanical strength without interfering with water movement from vessel to vessel. The largest vessel in poplar trees is near the base and the inside vessel diameter is between 30-120 µm; and the smallest vessel diameters are found close to the tree apex. This architecture is safer for the tree since smaller diameter vessels are less vulnerable to cavitation (Zimmermann, 1978; 1980). However, vessel diameter varies with the season, water availability and position within the stem (Mellerowicz, 2006).

### *2.5 Measurement of xylem-induced cavitation*

Leaf water supply and plant water use are influenced by factors affecting water transport, *i.e.*, xylem vulnerability to cavitation and xylem anatomy and the architecture and conductivity of the root system (Maherali *et al.*, 2006). The breakdown of the water column and the loss of conductivity occur by the entry of air through conduit pit membranes (cavitation), or disrupted by the entry of air sucked in through holes in the cell walls or pits (air seeding) resulting in embolism (Zimmermann, 1983; Meinzer *et al.*, 2001). Studies have shown that conductance is closely related to the vessel structure (e.g. pit membranes) and tends to decrease at low water potentials as a result of xylem embolism (Jones & Sutherland, 1993; Mauseth & Stevenson, 2004); where wider vessels are likely to cavitate faster than narrower vessels (Zimmermann, 1978; Salleo & LoGullo, 1986; Hacke & Sauter, 1996).

Cavitation has been studied in several poplars (Tyree *et al.*, 1994; Hacke & Sauter, 1996; Rood *et al.*, 2000; Hukin *et al.*, 2005) and different techniques have been described to analyze xylem embolism in poplars. In my study,

vulnerability to xylem cavitation was evaluated using the pressure vulnerability curves with the centrifuge technique. This technique was initially developed by Cochard *et al.* (2000), and subsequently modified by Cochard (2002). The principle of the technique is to generate negative pressure (*P*) and positive hydrostatic pressure differences ($\Delta P$) across the stem segment by spinning it in the centrifuge. If the ends of the sample are immersed in water, the pressure difference creates a water volume flow rate *F* (mmol $s^{-1}$) through the sample. The hydraulic conductance (*k*) is then expressed as:

$$k=F/\Delta P \qquad [2]$$

Vulnerability curves are then constructed based on the relationship between hydraulic conductance and xylem pressure (See Cochard, 2002). From these curves, a commonly used midpoint of pressure causing 50% ($P_{50}$) loss of conductivity can be calculated (Cochard *et al.*, 2007).

### *2.6 Plant mechanisms in response to drought*

Drought affect plants in many different ways (Boyer, 1982). Plant responses to drought stress are complex and involve a range of morphological, anatomical and physiological adjustments to control water loss and enhance water uptake (Yordanov *et al.*, 2003; Ennahej *et al.*, 2008). Early responses to water stress facilitate immediate survival, whereas acclimation, which compromises new metabolic and structural capabilities by altered gene expresion, helps to improve plant functioning under stress (Bohnert & Sheveleva, 1998). Survival mechanisms in response to water stress may include acclimation through metabolic or physiological changes, or temporary processes related to growth rate, plant structure, tissue osmotic potential, and stomatal and hydraulic conductances. In general, these mechanisms have been described as: (i) drought tolerance, maintenance of plant metabolism by stomatal control or by induction of adequate cell turgor due to osmoregulators, and (ii) drought avoidance, stress prevention by increasing water uptake and reducing water loss through the increase of stomatal resistance and changes in leaf area and anatomy (Touchette *et al.*, 2007).

Plant responses to drought differ at various organizational levels depending on the severity and duration of the stress, as well as the stage of plant development (Chaves *et al.*, 2003). Some of the responses to drought stress include synthesis and accumulation of solutes (e.g. sugars, amino acids) (Tschaplinski *et al.*, 1998; Tschaplinski & Tuskan, 1994; Watanabe *et al.*, 2000), regulation of aquaporins (Morillon & Lasalles, 2002; Lian *et al.*, 2004; Kaldenhoff *et al.*, 2008), stomatal regulation (Jackson *et al.*, 2000) and changes in anatomical characteristics associated with hydraulic conductance (Aasamaa *et al.*, 2001; Mauseth & Stevenson, 2004). Therefore, maintenance of water status or water flow under adverse conditions, requires the coordination of these and other parameters.

### *2.6.1 Role of osmoregulators in drought resistance*

Cell growth is one of the most drought-sensitive physiological processes due to the reduction in turgor pressure. Under severe water deficiency, cell elongation of higher plants can be inhibited by the interruption of water flow from the xylem to the surrounding elongating cells (Bradford & Hsiao, 1982; Nonami, 1998). In this sense, osmotic adjustment by accumulation of compatible solutes helps to maintain root and leaf turgor, as well stomata opening and carbon gain under low water potentials, without interfering with cellular structure or metabolism (Yancey *et al.*, 1982; Pereira & Pallardy, 1989; Nilsen & Orcutt, 1996). The main source of compatible solutes are either the photosynthates themselves or indirectly from the hydrolysis of carbon storage compounds. Thus, osmotic adjustment depends largely on the balance between carbon assimilation, allocation and utilization by various sinks (Blum, 1996).

Compatible solutes can play a role in drought-stressed plants by increasing water retention or reducing efflux from cells, therefore providing cell turgor (Bohner & Jensen, 1996; Hare *et al.*, 1998; Polle *et al.*, 2006). Osmotic adjustment leads to a decrease of osmotic potential and, as a consequence, increases the gradient for water influx into the cell, maintenance of turgor, and maintenance of photosynthetic activity at lower water potentials (Lawlor & Cornic, 2002). The functions of compatible solutes, include osmoprotection, which includes protection of thylakoid and plasma membrane integrity, stabilizing non-

membrane proteins, creating a sink for energy and reducing agents, providing a source of carbon and nitrogen for recovery, and scavenging of reactive oxygen species, which are partially reduced or activated derivates of oxygen molecules that can lead to oxidative destruction of cell (Hare *et al.*, 1998; Sairam *et al.*, 2006; Taiz & Zeiger, 2006).

There are four main classes of osmotically active solutes: inorganic ions, sugars, non-protein amino acids, and organic acids (Nilsen & Orcutt, 1996). Some of the most common osmoregulators reported in response to drought are: monosaccharides (fructose, glucose); disaccharides (sucrose); and amino acids (proline) (Arnd *et al.*, 2001; Gebre & Tschaplinski, 2002; Monreal *et al.*, 2007). Drought stress is accompanied by a shift in the partitioning of photosynthates, increasing levels of sucrose and/or reducing sugars that have been proposed to contribute towards the maintenance of turgor (Hare *et al.*, 1998). Mobilization of non-structural carbohydrates has been reported in cultivars resistant to drought (Yang *et al.*, 2004; Cuellar-Ortiz *et al.*, 2008). In poplars, accumulations of soluble carbohydrates and total free amino acids have also been reported (Tschaplinski & Blake, 1989; Tschaplinski & Tuskan, 1994; Gebre *et al.*, 1997; Tschaplinski *et al.*, 1998; Lei *et al.*, 2007). These studies suggest that lower osmotic potential allows the plant to withstand low soil water content and facilitate water movement from the soil to the plant.

Proline accumulation at low leaf water potential is caused by a combination of increased biosynthesis, regulated by enzyme activity and gene expression, and slower oxidation in mitochondria; this accumulation is controlled by transport and utilization rates (Delauney & Verma, 1993; Raymond & Smirnoff, 2002). Under drought, glutamate is the dominant precursor of proline via $\Delta^1$–pyrroline-5-carboxylate (P5C) and is catalyzed by P5C synthetase and P5C reductase (Samaras *et al.*, 1995; Raymond & Smirnoff, 2002). Several roles of proline under stress have been proposed, including stabilization of proteins and membranes, a sink for excess of reductants, and a store of carbon and nitrogen for use after relief from drought stress (Delauney & Verman, 1993; Monreal *et al.*, 2007). The role of proline as water structure regulator may be due to the induction of formation of strong H-bonded water around proteins preserving the native state of the cell biopolymers in water-stressed cells (Stewart & Hanson, 1980; Rascio *et al.*, 1994). In terms of time course of induction, proline synthesis

may display different patterns of expression, depending on whether it is for osmotic adjustment or for protection against cell injury (Delauney & Verma, 1993).

### *2.6.2 Stomatal regulation*

Stomata play an important role as a pressure regulator controlling plant water flow rate (Meinzer & Grantz, 1990; Sperry *et al.*, 2002). Stomatal control has been identified as an early response to drought stress; however different factors can alter stomatal responses (Chaves *et al.*, 2003). Stomatal closure under drought stress may involve chemical signals from roots to leaves (Rodrigues *et al.*, 2008), hydraulic signals (Christmann *et al.*, 2007) and changes in xylem conductance (Salleo *et al.*, 2000; Nardini *et al.*, 2001). Stomatal closure decreases internal carbon dioxide concentrations which, in turn, alter photosynthetic mechanisms in the short term (Nilsen & Orcutt, 1996; Cochard *et al.*, 1992).

Moreover, stomatal regulation in plants is important in terms of optimizing either survival or productivity since it may prevent the risk of xylem cavitation and, in the long term, stomatal closure contributes to an enhancement of water use efficiency (Jones & Sutherland, 1993; Tyree & Sperry, 1989; Jackson *et al.*, 2000; Liu *et al.*, 2005). In poplars, stomatal regulation has been correlated with drought resistance by preventing decreases in water potential below the threshold needed to induce cavitation (Blake *et al.*, 1984; Tschaplinski *et al.*, 1998). In these studies, more favorable water balance was related to the degree and duration of stomatal closure. Moreover stomatal regulation affects cavitation resistance by preventing the breakdown of the water column (Braatne *et al.*, 1992; Harvey & van den Driessche, 1997).

### *2.6.3 Resistance to xylem cavitation*

Cavitation induces decreases in hydraulic conductance at different levels; which affects plant productivity (e.g. Sperry *et al.*, 1993: Salleo *et al.*, 2000: Maherali *et al.*, 2006). Therefore, it is important to understand the mechanisms used by plants to prevent xylem embolism. Some studies suggest that inter-

conduit pits are the most important sites for air-seeding in the cavitation process (Sperry *et al.*, 1996; Domec *et al.*, 2006). Accordingly, adaptation to dry conditions may involve mechanisms that minimize the occurrence of cavitation, or mechanisms that minimize their detrimental consequences (Jones & Sutherland 1993). Some plants may modify some vessels for cavitation resistance by altering their pit membranes, lignifying cell walls of the xylem conduits, or by surrounding them with paratracheal parenchyma (Mauseth & Stevenson, 2004; Choat *et al.*, 2008). However, differences can be found between species and between individual vessels within one plant (Jones & Sutherland, 1993).

Resistance to cavitation has an important impact on the capacity of xylem elements to transport water in poplars. Poplars have several small vessels and few large vessels, which are more efficient water conductors than small vessels; however small vessels seems to be less vulnerable to cavitation (Grace, 1993; Harvey & Van Den Driessche, 1997; Zimmermann, 1983). In general, poplars do not rely on root pressure to refill cavitated vessels (Sperry *et al.*, 1994; Hacke & Sauter, 1996); therefore, the capacity of xylem resistance to cavitation and/or development of different refilling mechanisms in these trees may be crucial in determining their adaptation to drought.

### *2.7 Recovery mechanisms after drought*

Embolism repair was considered irreversible, however several studies had showed refilling mechanisms in leaves (Canny 2001; Lo Gullo *et al.*, 2003), roots (Tyree *et al.*, 1986; Sperry *et al.*, 1994), and stems and petioles (Zwieniecki & Holbrook, 1998; Hacke & Sperry, 2003). The refilling process will occur when the gas inside each conduit comes under a positive pressure, causing it to dissolve (Zimmermann, 1983). Several theories have been proposed to explain the refilling mechanism, such as (a) positive root pressure, (b) an osmoregulation mechanism, and (c) embolism refilling by dissolution of air. In the positive root pressure mechanism, the gas is squeezed out of the system under pressure as the sap rises in the vessels (Tyree & Zimmermann, 2002; Westhoff *et al.*, 2008). The osmoregulation mechanism described by Canny (1997), involves the changes in the starch-sugar balance providing a long term change in tissue

pressure during the night and stimulation of water uptake during the day through transpiration. A mechanism described by Salleo *et al.* (1996; 2004; 2006) involves the participation of living parenchyma cells in the refilling mechanism; and it also has been proposed that transmembrane transport of water from these cells to the vessels through water channels significantly contributes to the process (Sakr *et al.*, 2003). The refilling mechanism by air dissolution, initially proposed by Sperry *et al.*, (1987), and subsequently theoretically described by Yang & Tyree (1992), involves increases in xylem pressure ($P_x$) thus removing air bubbles into the liquid at the surface of the bubble. The dissolution of air bubbles is influenced by the air pressure of the bubbles and the radii meniscus (Yang & Tyree, 1992; Shen *et al.*, 2008). According to Henry's law, the pressure threshold that is needed for emboli to dissolve must exceed xylem pressure ($P_x = -2T/r$); where $T$ is the surface tension of water and $r$ is the radius of curvature of the air-water interface (Yang & Tyree, 1992; Tyree & Zimmermann, 2002).

A final refilling mechanism, described by Wagner *et al.* (2000), includes the participation of lipid deposits on the inner xylem walls, thus providing an efficient barrier against water loss during drought. These deposits are subsequently disintegrated, after re-watering, forming lipid bodies and vesicle-like structures. These structures might play an important role in the transpiration flow and in the water ascent in hydrated plants by generation of Marangoni streaming at their interfaces. The Marangoni effect, transference of water due to surface tension differences, might facilitate lipid removal upon re-watering (Schneider *et al.*, 2000).

### *2.8 Biology of poplars*

The genus *Populus* is a member of the *Salicaceae* family and includes six taxonomically distinct sections consisting of approximately 30 species widely distributed in the northern hemisphere, from about 22°N to 70°N latitude (Palmer, 1991). These species are placed taxonomically in six sections: *Abaso*, *Turanga*, *Aigeiros*, *Tacamahaca, Leucoides* and *Populus* (Dickmann, 2001). The ability of poplar species to adapt to a broad range of sites, in addition to other features, such as relatively fast growth, ease of vegetative propagation, desirability of fiber

and wood properties, and early flowering, have contributed to the increase of *Populus* plantations around the world (Hinckley *et al.*, 1989; Palmer, 1991).

Poplars are predominantly dioecious, with male (pollen-bearing) and female (seed-producing) flowers occurring on separate trees (Dickmann & Stuart, 1983). Reproduction is both sexual and vegetative (asexual) and is influenced by the ecosystem (Braatne *et al.*, 1999). Vegetative propagation has led to production of clones that are exact genetic copies of their parent (Dickmann, 2001). In addition, poplar trees display coppice re-growth, the production of additional shoots from stumps; these new shoots have higher growth capacity compared with the original tree (Rood & Mahoney, 1991). The new stump shoots are produced from shallow and horizontal roots (Dickmann, 2001).

The ecology and life strategies of poplars are dynamic, ensuring constantly changing patterns of forest distribution and abundance. Poplars generate hybrids by naturally and controlled pollinations (Hinckley *et al.*, 1989; Riemenschneider *et al.*, 2001). Natural and artificial hybridization is widely established in the northern hemisphere (Zasada & Phipps, 1990; Floate, 2004; Vanden Broeck *et al.*, 2005). In Canada, plantations of different hybrid poplars were established from the 1940's to the 1970's in several provinces (Palmer, 1991). Hybridizations between sections *Aigeiros* and *Tacamahaca* are the most common because of the ease of hybridization between their species (Dickmann, 2001; Cervera *et al.*, 2005; Hamzeh *et al.*, 2007). Natural hybridization of these two sections has been found in Alberta and British Columbia (Braatne, 1999; Floate, 2004).

Poplar hybrids have been widely established due to their rapid juvenile growth (often between 1 and 7+ m of annual height increment), vegetative reproduction, and capacity to supply a broad range of wood products (Stettler *et al.*, 1996; Rood *et al.*, 2003). Hybrid poplars have shown hybrid vigour, expressed in terms of superior height and biomass compared with their parents. This vigour is related to stomatal responsiveness to drought and leaf characteristics (e.g., cell size and number, leaf shape, growth and size) (Hinckley *et al.*, 1989). In relation to drought adaptations, hybrid poplars exhibit variation among species and clones (Blake *et al.*, 1996; Hinckley *et al.*, 1989; Tschaplinski *et al.*, 1998; Bonhomme *et al.*, 2008). This variability might be due to the fact that most poplar species exhibit leaf plasticity, where plants will shift their allocation

patterns in varying environments to increase acquisition of growth-limiting resources (Vanende, 1991; Hale *et al.*, 2005). In addition, several studies have demonstrated the significance of hybrid poplars in phytoremediation (Bois *et al.*, 2005; Zalesny Jr. *et al.*, 2006; Strycharz & Newman, 2009), and as carbon sinks (Braatne, 1999; Balatinecz & Kretschmann, 2001; Robinson *et al.*, 2007).

Poplar hybrids used in this study are members of the sections *Tacamahaca* and *Aigeiros.* The balsam poplars in the *Tacamahaca* section are generally adapted to wetter regions. Several species of this section are of commercial importance. This section includes nine species, where *Populus balsamifera* is the most widely and natural distributed in North America. *P. balsamifera* extends from the western or northern region to southern Alberta, under wet and cool conditions (Kranjcec *et al.*, 1998; Pearce *et al.*, 2005).

Poplars of the section *Aigerios* include some of the major riparian poplars in the northern hemisphere. Species of this section are of commercial significance, and are predominant in the warmest and driest eastern prairie ecoregions. In southern Alberta, *P. deltoides* Bartr. *ex.* Marsh extends along Prairie Rivers with warmer and drier climates (Kalischuk *et al.*, 2001; Pearce *et al.*, 2005). Optimum growth conditions for this species are usually associated with bottomlands, alluvial and riparian corridors, and in silty or sandy loam soils. Riparian corridors consist of coarse textured materials that have been deposited by the river; since these materials are highly permeable, water infiltration and drainage are rapid (Mahoney, 1996). These trees are also characterized by their intolerance to shade and high capacity to invade newly exposed wet soils (Dickmann, 2001).

### *2.8.1 Studied poplar clones*

**Balsam poplar *(P. balsamifera* L.*)***

Balsam poplar is one the most widely distributed tree species in the northern mixed-wood forest of North America, and is distributed throughout the boreal forest from Newfoundland to Alaska (Vanende, 1991). Optimal growth conditions for this poplar are usually on floodplains and on floodplain-associated sandy and silty soils (Zasada & Phipps, 1990), although the trees will also grow on drier and warmer sites (DesRochers *et al.*, 2004). Trees of this species have

been planted for shelterbelts due to its adaptability to dry conditions, resistance to cold, and ease of propagation by stem or root cutting (Dickmann & Stuart, 1983; Rood & Mahoney, 1991). A recent study showed the use of this species in phytoremediation (Huynh *et al.*, 2008). Balsam poplar has a good capacity for recovery after severe drought periods, and is exceptionally well adapted to survive the extreme cold climate of Alberta (Rood & Mahoney, 1991; Pearce *et al.*, 2005).

**Northwest [*P. balsamifera* x *P. deltoides* cv. Northwest (Jackii hybrid)]**

This male hybrid poplar clone is well adapted to wet conditions and is more vigorous than pure species cottonwoods. Northwest is an important clone in the northern prairies. These trees can grow in cold areas, but are susceptible to wind damage (Dickmann & Stuart, 1983; Eckenwalder, 2001). They are also sensitive to leaf rust (*Melampsora* spp.) (Chang *et al.*, 1989; Eckenwalder, 2001), stem canker (*Septoria musiva*) (Eckenwalder, 2001; LeBoldus *et al.*, 2009) and aphids (Roller *et al.*, 1972). This hybrid has also been shown to have vigorous initial growth, maintain high xylem pressure potentials, photosynthesis and transpiration rates (Tschaplinski & Blake, 1989a). In addition, Northwest trees are considered resistant to drought (Tschaplinski & Blake, 1989b), salt stress (Renault *et al.*, 1999), and respond well to elevated $CO_2$ concentrations (Tupker *et al.*, 2003).

**Walker (*P. deltoides* x *P.* x *petrowskyana* cv. Walker)**

*P.* x *petrowskyana* is a hybrid from *P. nigra* x *P. laurifolia*. Walker is a female hybrid clone which was distributed in 1963 to farmers in the prairies of Canada (Lindquist *et al.*, 1977). This clone is more suitable to grow in sandy loam, sandy clay loam and loam soils. Trees have a single main stem and narrowly ascending branches (Steppuhn *et al.*, 2008). Walker is an outstanding clone resulting in it's widespread distribution throughout the prairies for shelterbelts, and is widely used in the southern Canadian prairies (Lindquist *et al.*, 1977; Cram & Lindquist, 1982; Eckenwalder, 2001). It is also moderately tolerant to salinity, (Steppuhn *et al.*, 2008), resistant to leaf rust (Chang *et al.*, 1989) as well as many insects and diseases (Lindquist *et al.*, 1977), and is also

suitable to grow in the mixed grassland ecoregions, aspen parkland and boreal transition areas (Walker & Schroeder, 2001). This hybrid however, is less water efficient and exhibits less drought resistant compared with other hybrid poplars (DesRochers *et al.*, 2007). .

**P38P38 (*P. balsamifera* x *P. simonii* cv. P38P38)**

There have been relatively few studies describing the physiology of this hybrid poplar. This female clone as well as Northwest, responds well to elevated concentrations of $CO_2$ (Tupker *et al.*, 2003) and under field conditions, P38P38 showed greater drought resistance than Walker (DesRochers *et al.*, 2006).

**Okanese (Walker x *P. x petrowskyana* cv. Okanese)**

Okanese is a new clone released in spring 2008 by the Prairie Farm Rehabilitation Administration (PFRA) in Indian Head Saskatchewan. Okanese has shown to be efficient at recycling nutrients, making this tree suitable for long-term production (Booth, 2008). In addition, this hybrid has also shown moderate resistance to changes in temperature, maintaining patterns of consistent internal dormancy (Kalcsits, 2007).

**Assiniboine (open-pollinated Walker cv. Assiniboine)**

Assiniboine is a fast-growing and hardy male clone was available from the poplar improvement program of the PFRA (Schroeder & Linquist, 1989). This hybrid poplar is extensively used in plantations and shelterbelts of the Canadian prairies (PFRA Shelterbelt Centre, 2001), and exhibits high tolerance to salinity (Steppuhn *et al.*, 2008). Assiniboine prefers loam soils with a minimum of 300 mm of precipitation per year. These trees have shown resistance to extreme cold temperatures (up to -60°C). Growth rates of Assiniboine are similar to Walker and Northwest poplars (Schroeder & Linquist, 1989).

**Berlin *(P. x berolinensis* Dippel cv. Berlin*)***

This male hybrid originated spontaneously in the Berlin Botanical Garden in the late 1800s, from a cross between *P. laurifolia* x *P. nigra*. This cross is also known as *P.* x *petrowskyana* Schr. and *P.* x *rasumowskyana* Schr. Berlin poplar

has slow to moderate growth rates and can be planted on moderately dry sites. It is susceptible to canker (*Septoria musiva*), leaf rust (*Melampsora*) and aphids and is intolerant to early autumn and late spring frosts (Roller *et al.*, 1992). This tree has been used in North America for windbreaks, roadside plantings, as well as pulpwood (Dickmann & Stuart, 1983).

## References


Aasamaa K, Sõber A, Rahi M (2001) Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Aust J Plant Physiol* **28**, 765-774.

Angeles G, Bond B, Boyer JS, Brodribb T, Brooks JR, Burns MJ, Cavender-Bares J, Clearwater M, Cochard H, Comstock J, Davis SD, Domec J-C, Donovan L, Ewers F, Gartner B, Hacke U, Hinckley T, Holbrook NM, Jones HG, Kavanagh K, Law B, López-Portillo J, Lovisolo C, Martin T, Martínez-Vilalta J, Mayr S, Meinzer FC, Melcher P, Mencuccini M, Mulkey S, Nardini A, Neufeld HS, Passioura J, Pockman WT, Pratt RB, Rambal S, Richter H, Sack L, Salleo S, Schubert A, Schulte P, Sparks JP, Sperry J, Teskey R, Tyree M. 2004. The Cohesion-Tension Theory. *New Phytologist* 163, 451–452.

Arndt, SK, Clifford SC, Wanek W, Jones HG, Popp M (2001) Physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought stress. *Tree Physiol.* **21**, 705-715.

Aroca R, Amodeo G, Fernández-Illescas S, Herman EM, Chaumont F, Chrispeels MJ (2004) The role of aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. *Plant Physiol* **137**, 341-353

Balatinecz JJ, Kretschmann DE (2001) Properties and utilization of poplar wood. *In*: Poplar culture in North America. Part A. Chapter 9. Dickmann DI, Isebrands JG, Eckenwalder JE, and Richardson J (eds) NRC Research Press. National Research Council of Canada, Ottawa, ON. Pp. 277-291.

Baker, DA. (1984) Water relations. *In*: Advanced Plant Physiology. Wilkins MB (Ed). United Kingdom. Pp. 297-318.

Blake TJ, Bevilacqua E, Zwiazek JJ (1991) Effects of repeated stress on turgor pressure and cell elasticity changes in black spruce seedlings. *Can J For Res.* **21**, 1329-1333.

Blake TJ, Tschaplinski TJ, Eastman A (1984) Stomatal control of water use efficiency in poplar clones and hybrids. *Can J Bot* ..**62**:1344-1351

Blake TJ, Sperry JS, Tschaplinski TJ, Wang SS (1996) Water Relations. *In*: Biology of *Populus* and its Implications for Management and Conservation Part II, Chapter 16. Stettler RF, Bradshaw HD Jr, Heilman PE, Hinckely TM (Eds). NRC Research Press, National Research Council of Canada, Ottawa, ON. Pp 401–22.

Blum A (1996) Crop responses to drought and the interpretation of adaptation. *Plant Growth Regul* **20**, 135–148.

Bohnert HJ, Jensen RG (1996) Strategies for engineering water-stress tolerance in plants. *TIBTECH.* **14**, 89-97.

Bohnert HJ, Sheveleva E (1998) Plant stress adaptations – making metabolism move. *Curr Opin Plant Biol* **1**, 267-274.

Bois G, Piche Y, Fung MYP, Khasa DP (2005) Mycorrhizal inoculum potentials of pure reclamation materials and revegetated tailing sands from the Canadian oil sand industry. *Mycorrhiza* **15**, 149-158.

Bonhomme L, Barbaroux C, Monclus R, Morabito D, Berthelot A, Villar M, Dreyer E, Brignolas F (2008) Genetic variation in productivity, leaf traits and carbon isotope discrimination in hybrid poplars cultivated on contrasting sites. *Ann For Sci.* **65**, 503-

Booth NWH (2008) Nitrogen fertilization of hybrid poplar plantations in Saskatchewan, Canada. Master Thesis. Department of Soil Science University of Saskatchewan. Saskatoon, Saskatchewan, Canada. P. 117,

Bradford KJ, Hsiao TC (1982) Stomatal behavior and water relations of waterlogged tomato plants. *Plant Physiol.* **70**, 1508-1513.

Braatne JH, Hinckley TM, Stettler RF (1992) Influence of soil water on the physiological and morphological components of plant water balance in *Populus trichocarpa*, *Populus deltoides* and their F1 hybrids. *Tree Physiol.* **11**, 325-339.

Braatne JH, Rood SB, Heilman PE (1996) Life history, ecology, and reproduction of riparian cottonwoods in North America. *In*: Biology of Populus and its Implications for Management and Conservation. Stettler RF, Bradshaw HD, Jr, Heilman PE, Hinckley TM (Eds). Ottawa, Canada: NRC Research Press, 57–85.

Braatne J (1999) Biological aspects of hybrid poplar cultivation on floodplains in Western North America: review. US EPA 1999; Doc. no. 910-R-99-002.

Canny MJ (1997) Vessel contents during transpiration – embolisms and refilling. *Am J Bot* **84**, 1223-1230.

Canny MJ (2001) Embolism and refilling in the maize leaf lamina and the role of the protoxylem lacuna. *Am J Bot* **88**, 47-51.

Castro-Diez P, Navarro J (2007) Water relations of seedling of three *Quercus* species: variations across and within species grown in contrasting light and water regimes. *Tree Physiol.* **21**, 1011-1018.

Cervera MT, Storme V, soto A, Ivens B, Van Mongatu M, Rajora OP, Boerjan W (2005) Intraspecific and interespecific genetic and phylogenetic relationships in the genus *Populus* based on AFLP markers. *Theor Appl Genet* **111**, 1440-1456.

Chang KF, Hwang SF, Neuwirth ME (1989) Incidence and severity of *Melampsora* leaf rust of poplar grown under controlled conditions. *Can Plant Dis Surv* **69**, 121-124.

Chaves MM (1991) Effects of water deficits on carbon assimilation. *J Exp Bot* **42**, 1-16.

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML, Caravalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field. Photosynthesis and growth. *Annals Bot* **89**, 907-916.

Chaves MM, Maroco JP, Pereira JS (2003) Review: Understanding plant responses to drought – from genes to the whole plant. *Func Plant Biol* 30, 239-264.

Choat B, Cobb AR, Jansen S (2008) Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function. *New Phytol* **177**, 608-626.

Christmann A, Weiler E, Steudle E, Grill E (2007) A hydraulic signal in root-to-shoot signalling of water shortage. *Plant J* **52**, 167-174.

Cochard H, Cruiziat P, Tyree MT (1992) Use of positive pressures to establish vulnerability curves: further support for the air-seeding hypothesis and implications for pressure-volume analysis. *Plant Physiol* **100**, 205-209.

Cochard H, Bodet C, Ameglio T, Cruiziat P (2000) Cryo-scanning electron microscopy observation of vessel content during transpiration in walnut petioles. Facts of artifacts? *Plant Physiol* **124**, 1191-1202.

Cochard H, Forestier S, Ameglio T (2001) A new validation of the Scholander pressure chamber technique base on stem diameter variations. *J Exp Bot* **52**, 1361-1365.

Cochard H (2002) A technique for measuring xylem hydraulic conductance under high negative pressures. *Plant Cell Env* **25**, 815-819.

Cochard H, Barigah ST, Kleinhentz M, Eshel A (2007) Is xylem cavitation resistance a relevant criterion for screening drought resistance among *Prunus* species? *J Plant Physiol* **165**, 976-982.

Cram WH, Lindquist CH (1982) Overwinter storage of Walker poplar cuttings. *For Chron* 176-177.

Cuellar-Ortiz SM, Arrieta-Montiel M, Acosta-Gellegos J, Covarrubias AA (2008) Relationship between carbohydrate partitioning and drought resistance in common bean. *Plant Cell Environ.* **31**, 1399-1409.

Delauney AJ, Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. *Plant J* **4**, 215-223.

DesRochers A, Thomas BR, Butson R (2004) Reclamation of roads and landings with balsam poplar cuttings. *For Ecol Manag* **199**, 39-50.

DesRochers A, van den Driessche R, Thomas BR (2006) NPK fertilization at planting of three hybrid poplar clones in the boreal region of Alberta. *For Ecol Manag* **232**, 216-225.

DesRochers A, van den Driessche R, Thomas BR (2007) The interaction between nitrogen source, soil pH, and drought in the growth and physiology of three poplar clones. *Can J Bot* ..**85**, 1046-1057.

Dickmann DI, Stuart KW (1983) The culture of poplars in eastern North America. Department of forestry. Michigan State University. Pp. 1-55.

Dickmann DI (2001) An overview of the genus Populus. *In:* Poplar culture in North America. Part A, Chapter 1. Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J (Eds). NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada. Pp. 1-42.

Dixon HH, Joly J (1894) On the ascent of sap. *Annals Bot* **8**, 468–470.

Domec J-C, Lachenbruch B, Meinzer FC (2006) Bordered pit structure and function determine spatial patters of air-seeding thresholds in xylem of Douglas-fir (*Pseudotsuga menziesii*; Pinaceae) trees. *Am J Bot* **93**, 1588-1600.

Eckenwalder JE (2001). Descriptions of clonal characteristics. In: Poplar Culture in North America. Part B, Chapter 13. Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J (Eds). NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada. Pp. 331-382.

Ennajeh M, Tounekti T, Vadel AM, Khemira H, Cochard H (2003) Water relations and drought-induced embolism in olive (*Olea europea*) varieties 'Meski' and 'Chemlali' during severe drought. *Tree Physiol.* **28**, 971-976.

Floate KD (2004) Extent and patterns of hybridization among the three species of *Populus* that constitute the riparian forest of the southern Alberta, Canada. *Can J Bot* ..82, 253-264.

Gardner WR, Rawlins SL (1965) Sap pressure in plants. *Science.* **149**, 920.

Grace J (1993) Consequences of xylem cavitation for plant water deficits. *In*: Water deficits plant responses from cell to community. Smith JAC & Griffiths H (Eds). Bios Scientific publishers. Oxford, UK.

Gebre GM, Brandle JR, Kuhns MR (1997) Influence of rewatering and time of sampling on solute accumulation of two *Populus deltoides* clones. *Tree Physiol.* **17**, 341-346.

Gebre GM, Tschaplinski TJ (2002) Solute accumulation of chestnut oak and dogwood leaves in response to throughfall manipulation of an upland oak forest. *Tree Physiol.* **22**, 251-260.

Hacke U, Sauter JJ (1996) Xylem dysfunction during winter and recovery of hyudraulic conductivity in diffuse porous and ring-porous trees. *Oecologia* **105**, 435-439.

Hacke U. Sperry JS (2003) Limits to xylem refilling under negative pressure in *Laurus nobilis* and *Acer negundo*. *Plant Cell Env* **26**, 303–311.

Hale BK, Herms DA, Hansen RC, Clausen TP, Arnold D (2005) Effects of drought stress and nutrient availability on dry matter allocation, phenolic glycosides, and rapid induced resistance of poplar to two lymantriid defoliators. *J Chem Ecol* **31**, 2601-2620.

Hamzeh M, Sawchyn C, Perinet P, Dayanandan S (2007) Asymmetrical natural hybridization between *Populus deltoides* and *P. balsamifera* (Salicaceae). *Can J Bot* ..**85**, 1227-1232.

Hare PD, Cress WA, Van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ.* **21**, 535-553.

Harvey HP, Van Den Driessche R (1997) Nutrition, xylem cavitation and drought resistance in hybrid poplar. *Tree Physiol.* **17**, 647-654.

Hinckley TM, Ceulemans R, Dunlap JM, Figliola A, Heilman PE, Isebrands JG, Scarascia-Mugnozza G, Scheulte PJ, Smit B, Stettler RF, Van Vokenburgh E, Wiard BM (1989) Physiological morphological and anatomical components of hybrid vigor in *Populus*. *In*: Structural and functional responses to environmental stresses. Kreeb KH, Richter H, Hinckley TM (Eds). SPB Academic Publishing. The Hague, The Netherlands. Pp. 199-217.

Huynh TT, Laidlaw WS, Singh B, Gregory D, Baker AJM (2008) Effects of phytoextraction on heavy metal concentrations and pH of pore-water of biosolids determined using an *in situ* sampling technique. *Environ Pollution* **156**, 8740882.

Hunking D, Cochard H, Dreyer E, Le Thiec D, Bogeat-Triboulot MB (2005) Cavitation vulnerability in roots and shoots: *does Populus euphratica* Oliv., a poplar from arid areas of Central Asia, differ from other poplar species? *J Exp Bot* **56**, 2003-2010.

Jackson RB, Sperry JS, Dawson TE (2000) Root water uptake and transport: using physiological processes in global predictions. *Trends Plant Sci* **5**, 482-488.

Jones HG, Sutherland RA. (1993) Stomatal control of xylem cavitation. *In*: Water Transport in Plants under climatic stress. Borghetti M, Grace J, Raschi A (Eds). Cambridge University Press, Pp. 41-51.

Kalcsits LA (2007) Exploring how temperature affects dormancy induction and cold acclimation initiation in hybrid poplar. Master Thesis. Department of Plant Science University of Saskatchewan. Saskatoon, Saskatchewan, Canada. 113 p.

Kalischuk AR, Rood SB, Mahoney JM (2001) Environmental influences on seedling growth of cottonwood species following a major flood. *Forest Ecol Manage* **144**, 75-89.

Kaldenhoff R, Ribas-Carbo M, Sans JF, Lovisolo C, Heckwolf M, Uehlein N (2008) Aquaporins and plant water balance. *Plant Cell Environ.* **31**, 658-666.

Kirkham MB (2005) Principles of soil and plant water relations. Elsevier Academic Press. USA.

Kranjcec J, Mahoney JM, Rood SB (1998) The responses of three riparian cottonwood species to water table decline. *For Ecol Manage* **110**, 77-87.

Kramer PJ (1969) Plant and soil water relationships: A modern synthesis. McGraw-Hill. USA.

Kramer PJ, Boyer J (1995) Water relations of plants and soils. Academic Press, San Diego. USA.

Lawlor DH, Corninc G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ.* **25**, 274-294.

LeBoldus JM, Blenis PV, Thomas BR, Feau N, Bernier L (2009) Susceptibility of *Populus balsamifera* to *Septoria musiva*: A field study and greenhouse experiment. *Plant Dis* **93**, 1146-1150.

Lei Y, Yin C, Li C (2007) Adaptive response of *Populus przewalskii* to drought stress and SNP application. *Acta Physiol Plan* **25**, 519-526.

Lemcoff JH, Ling F, Neumann PM (2006) Short episodes of water stress increase barley root resistance to radial shrinkage in a dehydrating environment. *Physiol Plant* **127**, 603-611.

Lian H-L, Yu X, Ye Q, Ding X-S, Kitagawa Y, Kwak S-S, Su W-A, Tang Z-C (2004) The role of aquaporins RWC3 in drought avoidance in rice. *Plant Cell Physiol* **45**(4), 481-489.

Lindquist CR, Cram WH, Howe JAG (1977) Walker poplar. *Can J Plant Sci* **57**,1019.

Liu F, Andersen MN, Jacobsen SE, Jensen CR (2005) Stomatal control and water use efficiency of soybean (*Glycine max* L. Merr.) during progressive soil drying. *Environ Exp Bot* **54**, 33-40.

Lo Gullo MA, Nardini A, Trifilio P, Salleo S (2003) Changes in leaf hydraulic and stomatal conductance following drought stress and irrigation in Ceratonia siliqua (Carob tree). *Physiol Plantarum* **117**, 186-194.

Maherali H, Moura CF, Caldeira MC, Willson CJ, Jackson RB (2006) Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees. *Plant Cell Environ.* **29**, 571-583.

Mahoney JM, Rood SB (1998) Streamflow requirements for cottonwood seedling recruitment – an integrative model. *Wetlands* ***18,634–645.***

Maseda PH, Fernández RJ (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. *J Exp Bot* **57**, 3963-3977.

Mauseth JD, Stevenson JF (2004) Theoretical considerations of vessel diameter and conductive safety in populations of vessels. *Int J Plant Sci* **165**, 359-368.

Meinzer FC, Grantz DA (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. *Plant Cell Environ.* **13**, 383–388

Meinzer FC, Clearwater MJ, Goldstein G (2001) Water transport in trees: current perspectives, new insights and some controversies. *Env Exp Bot* **35**, 239-262.

Mellerowicz EJ (2006) Xylem cell expansion – Lessons from Poplar. *In*: The science and lore of the plant cell wall: biosynthesis, structure and function. Hayashi T (Ed). BrownWalker Press. Boca Raton, Florida. Pp. 267-275.

Milburn JA (1996) Sap ascent in vascular plants: Challengers to the cohesion theory ignore the significance of immature xylem and the recycling of Münch water. *Annals Bot* **78**, 399-407.

Monreal JA, Jiménez ET, Remesal E, Morillo-Velarde R, García-Mauriño S, Echevarría (2007) Proline content of sugar beet storage roots: response to water deficit and nitrogen fertilization at field conditions. *Environ Exp Bot* **60**, 257-267.

Morillon R, Lasalles J-P (2002) Water deficit during root development: effects of the growth of roots and osmotic water permeability of isolated roots protoplasts. *Planta* **214**, 392-399.

Nardini A, Tyree MT, Salleo S (2001) Xylem cavitation in the leaf of *Prunus laurocerasus* and its impact on leaf hydraulics. *Plant Physiol* **125**, 1700-1709.

Nilsen ET, Orcutt DM (1996) Water limitation. *In*: Physiology of plants under stress. Abiotic factors. John Wiley and Sons (Eds). Pp. 322-361.

Nonami H (1998) Plant water relations and control of cell elongation at low water potentials. *J Plant Res* **111**, 373-382.

Palmer CL (1991) Short-rotation Culture of *Populus* and *Larix*: a literature review. Forest Resource Development Agreement. Ontario – Canada.

Passioura JB (1996) Drought and drought tolerance. *Plant Growth Regul* **20**, 79-83.

Pearce DW, Millard S, Bray DF, Rood SB (2005) Stomatal characteristics of riparian poplar species in a semi-arid environment. *Tree Physiol.* 26,211–218

Pereira JS, Pallardy S (1989) Water stress limitations to tree productivity. *In*: Biomass production by fast-growing trees. Pereira JS & Landsberg JJ (Eds). Kluwer Academic Publishers. NATO ASI series. Series E, Applied Sciences: vol. 166.

Polle A, Altman A, Jiang X (2006) Towards genetic engineering for drought tolerance in trees. *In*: Tree Transgenesis. Fladung M. & Ewald D. (Eds). Springer-Verlag Berlin Heidelberg. Pp. 275-297.

Prairie Farm Rehabilitation Administration (PFRA) (2001) Growing hybrid poplars as a crop. Canada-Saskatchewan Agri-Food Innovation fund and Prairie Farm Rehabilitation Administration, PFRA Factsheet. PFRA Shelterbelt Centre, Agriculture and Agri-Food Canada, Indian Head, SK 6 p.

Rascio A, Platini C, Scalfati G, Tonti A, Di Fonzo N (1994) The accumulation of solutes and water binding strength in durum wheat. *Physiol Plant* **90**, 715-721.

Raymond MJ, Smirnoff N (2002) Proline metabolism and transport in maize seedlings at low water potential. *Annals Bot* **89**, 813-823.

Redfield EB, Zwiazek JJ (2002) Drought tolerance characteristics of black spruce (*Picea mariana*) seedlings in relation to sodium sulfate and sodium chloride injury. *Can J Bot* ..**80**, 773-778.

Renault S, Paton E, Nilsson G, Zwiazek JJ, MacKinnon MD (1999) Responses of boreal plants to high salinity oil sands tailings water. *J Environ Qual* **28**, 1957-1962.

Riemenschneider DE, Berguson WE, Dickmann DI, Hall RB, Isebrands JG, Mohn CA, Stanosz GC, Tuskan GA (2001) Poplar breeding and testing strategies in the north central U.S.: Demonstration of potential yield and consideration of future research needs. *For Chron* **77**, 245-253.

Ritchie GA, Hinckley TM (1975) The pressure chamber as an instrument for ecological research. *Adv Ecol Res* **9**, 165-254.

Robinson BH, Green SR, Chancerel B, Mills TM, Clothier BE (2007) Poplar of the phytomanagement of boron contaminated sites. *Environ Pollution* **150**(2), 225-233.

Rodrigues ML, Santosa TP, Rodrigues AP, Souza CR, Lopes CM, Maroco JP, Pereira JS, Chaves MM (2008) Hydraulic and chemical signaling in the regulation of stomatal conductance and plant water use in field grapevines growing under deficit irrigation. *Functional Plant Biol* **35**, 565-579.

Roller KJ, Thibault DH, Hildahl V (1972) Guide to the identification of poplar cultivar on the prairies. Department of the Environment. Canadian Forestry Service. Publication No. 1311. Ottawa. 58 p.

Rood SB, Mahoney JM (1991) The concern of the riparian cottonwoods of southern Alberta. *In* The biology and management of southern Alberta's cottonwoods. Rood SB & Mahoney JM (Eds). University of Lethbridge, Alberta. Canada, Pp.1-10.

Rood SB, Patiño S, Coombs K, Tyree MT (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees* **14**, 248-257.

Rood SB, Braatne JH, Hughes FMR (2003) Ecophysiology of riparian cottonwoods: stream flow dependency, water relations and restoration. *Tree Physiol.* **21**, 1113-1124.

Sairam RK, Tyagi A, Chinnusamy V (2006) Salinity tolerance: cellular mechanisms and gene regulation. *In*: Plant Environment Interactions, 3rd ed. Huang B. (Ed). CRC Press, Boca Raton, FL.

Saito T, Terashima I (2004) Reversible decreases in the bulk elastic modulus of mature leaves of deciduous *Quercus* species subjected to two drought treatments. *Plant Cell Env* **27**, 863-875.

Sakr S, Alves G, Morillon R, Maurel K, Decourteix M, Guilliot A, Fleurat-Lessard P, Julien JL, Chrispeels MJ (2003) Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiol* **133**, 630-641.

Salleo S, LoGullo MA (1986) Xylem cavitation in nodes and internodes of whole *Chorisia insignis* H.B. et K. plants subjected to water stress: relations between xylem conduit size and cavitation. *Ann Bot* **58**, 431-441

Salleo S, Lo Gullo MA, De Paoli M, Zippo M (1996) Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. *New Phytol* **132**, 47-56.

Salleo S, Nardini A, Pitt F, Lo Gullo MA (2000) Xylem cavitation and hydraulic control of stomatal conductance in laurel (*Laurus nobilis* L.). *Plant Cell Environ.* **23**, 71-79.

Salleo S, Lo Gullo MA, Trifilò P, Nardini A (2004) New evidence for a role of vessel-associated cells and phloem in the rapid xylem refilling of cavitated stems of *Laurus nobilis* L. *Plant Cell Environ.* **27**, 1065-1076.

Salleo S, Trifilò P, Lo Gullo MA (2006) Phloem as a possible major determinant of rapid cavitation reversal in stems of *Laurus nobilis* (laurel). *Funct Plant Biol* **33**, 1063–1074

Samaras Y, Bressan RA, Csonka LN, Garcia-Rios M, Paino D'Urzo M, Rhodes D (1995) Proline accumulation during water deficit. *In*: Environment and plant metabolism. Flexibility and acclimation. Smirnoff N (Ed). Oxford: Bios Scientific Publishers.

Schneider H, Wistuba N, Wagner HJ, Thürmer F, Zimmermann U (2000) Water kinetics in refilling xylem after desiccation in a resurrection plant. *New Phytol* **148**, 221-238.

Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. *Science* **148**, 339-346.

Schroeder WR, Lindquist CH (1989) Assiniboine poplar. *Can J Plant Sc.* **68**, 351-353.

Schulze ED, Beck E, Müller-Hohenstein K (2005) Plant Ecology. Springer-Verlag. Berlin. pp. 702.

Shen FY, Guo R, Sun Q, Gao RF, Shen YB, Zhang ZY (2008) Possible causes for embolism repair in xylem. *Env Exp Bot* **62**, 139-144.

Siemens JA, Zwiazek JJ. 2004. Changes in water flow properties of solutions culture-grown trembling aspen (*Populus tremuloides*) seedlings under different intensities of water-deficit stress. *Physiol Plantarum* **121**, 44–49.

Sperry JS, Holbrook NM, Zimmermann MH, Tyree MT (1987) Spring filling of xylem vessels in wild grapevine. *Plant Physiol* **83**, 414–417.

Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *J Exp Bot* **44**, 1075–1082.

Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE (1994) Xylem embolism in ring-porous, diffuse-porous, and conferous trees of northern Utah and interior Alaska. *Ecology* **75**, 1736-1752.

Sperry JS, Saliendra NZ, Pockman WT, Cochard H, Cruiziat P, Davis SD, Ewers FW, Tyree MT (1996) New evidence for large negative xylem pressures and their measurement by the pressure chamber method. *Plant Cell Environ.* **19**, 427-436.

Sperry JS, Hacke UG, Oren R, Comstock JP (2002) Water deficits and hydraulic limits to leaf water supply. *Plant Cell Environ.* **25**, 251-263.

Steppuhn H, Kort J, Wall KG (2008) First year growth response of selected hybrid poplar cuttings to root-zone salinity. *Can J Plant Sci* **88**, 473-483.

Steudle E, Paterson CA (1998) How does water get through roots? *J Exp Bot* **49**, 775-788.

Steudle E (2000a) Water uptake by plant roots: an integration of views. *Plant Soil* **226**, 45-56.

Steudle E (2000b) Water uptake by roots: effects of water deficit. *J Exp Bot* **51**, 1531-1542.

Steudle E (2001) The cohesion-tension mechanism and the acquisition of water by plant roots. *Annual Rev. Plant Physiol Mol Biol* **52**, 847-875.

Steudle E, Ranathunge K (2007) Apoplastic water transport in roots. *In*: The apoplast of higher plants: Compartment of Storage. Sattelmacher B & Horst WJ (Eds). Springer. Dordrecht, The Nertherlands.

Stettler RF, Bradshaw HD, Jr Heilman PE, Hinckley TM (1996) Biology of Populus and its implications for management and conservation. NRC Research Press, Ottawa. 539 p.

Stewart CR, Hanson AD (1980) Proline accumulation as a metabolic response to water stress. *In*: Adaptation of plants to water and high temperature stress. Turner NC & Kramer PJ (Eds). John Wiley and Sons, New York, pp. 173-189.

Strycharz S, Newman L (2009) Use of native plants for remediation of trichloroethylene: I. Deciduous trees. *International J. Phytoremediation* **11**,150-170.

Taiz L, Zeiger E (2006) Plant Physiology. Fourth edition.

Tschaplinski TJ, Blake TJ (1989a) Water relations, photosynthetic capacity, and root/shoot partitioning of photosynthates as determinants of productivity in hybrid poplar. *Can J Bot* ..**67**, 1689-1697.

Tschaplinski TJ, Blake TJ (1989b) Water stress tolerance and late-season organic solute accumulation in hybrid poplar. *Can J Bot* .**67**, 1681-1688.

Tschaplinski TJ, Tuskan GA (1994) Water-stress tolerance of black and eastern cottonwood clones and four hybrid progeny. II. Metabolites and inorganic ions that constitute osmotic adjustment. *Can J For Res.* **24**, 681-687.

Tschaplinski TJ, Tuskan GA, Gebre GM, Todd DE (1998) Drought resistance of two hybrid *Populus* clones grown in a large-scale plantation. *Tree Physiol.* **18**, 653-658.

Touchette BW, Lannacone LR, Turner GE, Frank AR, (2007) Drought tolerance versus drought avoidance: a comparison of plant–water relations in herbaceous wetland plants subjected to water withdrawal and repletion. *Wetlands* **27**, 656–667.

Turner NC, Jones MM, (1980) Osmotic adjustment in expanding and fully expanded leaves of sunflower in response to water deficits. *Aust J Plant Physiol* **7**, 181–192.

Turner NC (1988) Measurement of Plant Water Status by the Pressure Chamber Technique. *Irrigation Sci* **9**, 289-308.

Tupker KA, Thomas BR, Macdonald SE (2003) Propagation of trembling aspen and hybrid poplar for agroforestry: potential benefits of elevated $CO_2$ in the greenhouse. *Agroforestry Systems.* **59**, 61-71.

Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *J. Exp. Bot.* **23**, 267-282

Tyree MT, Fiscus EL, Wullschleger SD, Dixon MA (1986) Detection of xylem cavitation in corn under field conditions. *Plant Physiol.* **82**, 597-599.

Tyree MT, Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. *Ann. Rev. Plant Phys. Plant Mol. Biol.* **40**, 19–38.

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New Phytol* **119**, 345-360.

Tyree MT, Kolb KJ, Rood SB, Patiño S (1994) Vulnerability to drought-induced cavitation of riparian cottonwoods in Alberta: a possible factor in the decline of the ecosystem? *Tree Physiol.* **14**, 455-466.

Tyree MT, Zimmermann MH (2002) Xylem Structure and the Ascent of Sap. Springer, Berlin.

Tyree MT (2003) Hydraulic properties of roots. *In*: Ecological Studies. H dr Kroon, WJW Visser (Eds). Root Ecology. Springer-Verlag berlin Heidelberg. Pp.125-150.

Van den Honert, TH (1948). Discuss. *Faraday Soc.* 3146-3153.

Vanden Broeck A, Villar M, Van Bockstaele E, Van Slycken J (2005) Natural hybridization between cultivated poplars and their wild relatives: evidence and consequences for native poplar populations. *Ann For Sci.* **62**, 601-613.

Vanende A (1991) Native poplar hybrids of Alberta – Analysis of balsam poplar species using flavonoid markers. *In*: The biology and management of southern Alberta's cottonwoods. Rood SB, Mahoney JM. (Eds). University of Lethbridge, Alberta. Canada, 69-78.

Walker DW, Schroeder WR (2001) Clonal evaluation of hybrid poplar on the canadian prairies. *In:* Temperate agroforestry: Adaptive and mitigative roles in a changing physical and socioeconomic climate. Schroeder W & Kort J (Eds). *Proc. Conf. Agrofor.* N.A. & Conf. Plains Prairie For. Assoc. Aug 13-15, 2001. Regina, Sk. Pp. 301-310.

Wagner HJ, Schneider H, Mimietz S, Wistuba N, Rokitta M, Krohne G, Haase A, Zimmermann U (2000) Xylem conduits of a resurrection plant contain a unique lipid lining and refill following a distinct pattern after desiccation. *New Phytol* **148**, 239-255.

Watanabe S, Kojima K, Ide Y, Sasaki S (2000) Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica* in vitro. *Plant Cell, Tissue and Organ Culture*. **63**, 199-206

Westhoff M, Shneider H, Zimmermann D, Mimietz S, Stinzing A, Wegner LH, Kaiser W, Krohne G, Shirley St, Jakob P, Bamberg E, Bentrup F-W, Zimmermann U (2008) The mechanisms of refilling of xylem conduits and bleeding of tall birch during spring. *Plant Biol.* **10**, 604-623.

Wiedenhoeft AC, Miller RB (2005) Structure and function of wood. *In*: Handbook of wood. Chemistry and wood composites. Rowell RM (Ed). CRC Press. Boca Raton, Fla.

Yancey, P.H., M.E. Clark, S.C. Hand, R.D. Bowlus and G.N. Somero, 1982. Living with water stress: Evolution of osmolyte system. *Science* **217**, 1214-1222.

Yang S, Tyree MT (1992) A theoretical model of hydraulic conductivity recovery from embolism with comparison to experimental data on *Acer saccharum*. *Plant Cell Environ.* **15**, 633-643.

Yang J, Zhang J, Wang Z, Zhu Q, Liu L (2004) Activities of fructan- and sucrose-metabolizing enzymes in wheat stems subjected to water stress during grain filling. *Planta.* **220**, 331-343.

Yordanov I, Velikova V, Tsonev T (2003) Plant response to drought and stress tolerance. *Bulg. J. Plant Physiol.* Special Issue. 187-206.

Zalesny Jr. RS, Wiese AH, Bauer EO, Riemenschneider DE (2006) Sapflow of hybrid poplar (*Populus nigra* L. x *P. maximowiczii* A. Herny 'NM6') during phytoremediation of landfill leachate. *Biomass Bioenergy*. **20**, 784-793.

Zasada JC, Phipps HM (1990) *Populus balsamifera* L. *In:* Silvics of North America: 2. Hardwoods. Agriculture Handbook 654. Burns RM, Honkala BH (Eds.) USDA, Forest Service, Washington, DC. Pp. 1019-1043.

Zimmermann MH (1978) Hydraulic architecture of some diffuse-porous. *Can J Bot* .**56**, 2286-2295

Zimmermann MH (1980) Transport in the xylem. *In*: Trees structure and function. Zimmermann MH & Brown CL (Eds). Springer-Verlag. Berlin Heidelberg - New York. Pp. 169-216.

Zimmermann MH (1983) Xylem structure and the ascent of sap. Springer, New York.

Zwieniecki MA, Holbrook NM (1998) Short term changes in xylem water conductivity in white ash, red maple and white spruce. *Plant Cell Environ.*. **21**, 1173-1180

# Chapter Three

## Leaf water relations and vessel resistance to xylem cavitation

### *3.1 Introduction*

Drought is an environmental constraint to plant survival and productivity (Boyer, 1982). Climate analysis and projections for the Western Prairie Provinces of Canada indicate increased variability of precipitation amount, intensity and frequency due to climate change (Schindler & Donahue, 2006; Lemprière *et al.*, 2008). Increases in the length and intensity of warming periods lead to changes in the hydrological cycle, accelerating land surface drying and increasing the potential of incidence and severity of drought (Huntington, 2006; Trenberth, 2007). These environmental changes may affect commercially important tree species such as poplars which are considered to be relatively water-demanding and drought-sensitive (Tyree *et al.*, 1994; Souch & Stephens, 1998).

Drought may lead to xylem cavitation, air- and vapour-filled conduits that no longer carry water, thus decreasing xylem hydraulic conductivity (Tyree & Ewers, 1991). Plants use stomatal closure as the main mechanism preventing excessive loss of water and xylem cavitation (Bond & Kavanagh, 1999; Cochard *et al.*, 2002). Several studies also suggest that the hydraulic architecture of the xylem is also involved in cavitation resistance. It has been noted that plants from arid environments are more resistant to cavitation compared with plants adapted to mesic environments (Pockman & Sperry 2000).

Given that plants cannot always avoid cavitation, they have developed mechanisms to alleviate its effects (Sperry, 1995). Some studies suggest that cavitated vessels can be refilled by several mechanisms including root pressure (Westhoff *et al.*, 2008), secretion of salts/solutes from the adjacent living cells to embolized vessels (Holbrook & Zwieniecki, 1999), flow of solutes through water channels (Tyree *et al.*, 1999), or activation of aquaporin genes in the xylem parenchyma (Sakr *et al.*, 2003). There is also evidence of functional coordination between vulnerability to xylem cavitation and the regulation of stomatal conductance across species, maintaining water potential above the range that would lead to cavitation (Sparks & Black, 1999; Maherali *et al.*, 2006). This

mechanism has been found in several woody species (Nardini *et al.*, 2000; Sperry & Hacke, 2002; Tyree *et al.*, 2003) suggesting that plants with higher capacity for xylem hydraulic conductance under water deficit stress also have a better chance of survival. Although poplars have been reported to be relatively susceptible to xylem cavitation (Tyree *et al.*, 1994; Hacke & Sauter, 1995; Rood *et al.*, 2000; Hukin *et al.*, 2005), there is little information about stomatal regulation in relation to xylem vulnerability to cavitation in poplars.

In the present study, I have compared drought responses in rooted shoot cuttings of six hybrid poplar clones and balsam poplar to determine the relationship between stem resistance to xylem cavitation, stomatal characteristics and conductance, and water potential. The clones were selected due to their commercial importance (Dickmann, 2001), and wide range of drought stress responses (DesRochers, *et al.*, 2007; Silim *et al.*, 2009). In the present study, I subjected plants to mild drought, severe drought (by withholding watering), and drought recovery (rewatering) treatments and measured leaf and stem water potentials and stomatal conductance. I also determined the vulnerability of shoot to cavitation using the Cavitron technique to explain the differences in stomatal responses to drought between the clones.

## *3.2 Materials and Methods*

### *3.2.1 Plant material and greenhouse conditions*

Ten-cm-long stem cuttings of P38P38 (*P. balsamifera* x *P. simonii* cv. P38P38), Walker (*P. deltoides* x (*P.* x *petrowskyana*) cv. Walker), Okanese (Walker x (*P. x petrowskyana*) cv. Okanese), Northwest [*P. balsamifera* x *P. deltoides* cv. Northwest (Jackii hybrid)], Assiniboine (open-pollinated Walker cv. Assiniboine), Berlin (*P. x berolinensis* cv. Berlin), and balsam poplar (*P. balsamifera* L.) were collected in February 2006 from Alberta-Pacific Forest Industries Inc. (Al-Pac) experimental field site (54°49'N, 113°31'W), located about 200 km northeast of Edmonton, Alberta, Canada. The cuttings were transported to the University of Alberta where they were soaked in water at room temperature for two days prior to planting (DesRochers & Thomas, 2003). Each cutting was

rooted in Metro Mix® 290 (Sun Gro Horticulture, Vancouver, BC, Canada ™) growing media in Styroblocks (440 ml volume/cell) (Beaver Plastic Ltd. Alberta, Canada) for 45 days and fertilized weekly with a 200 mg $L^{-1}$ solution of 15-30-15 (N-P-K) fertilizer (Plant Products Company Ltd., Brampton Ont.). Rooted cuttings were transferred to 4.5 L (6" x 7") plastic pots filled with homogenized Metro Mix® 290 amended with 2.73 $gL^{-1}$ Nutricote® 13-13-13 100 days slow-release fertilizer (6.5% $NO_3$-N, 6.5 $NH_4$-N, 13% $P_2O_5$, 13% $K_2O$, 1.2% Mg, 0.02% B, 0.05% Cu, 0.2% chelated Fe, 0.06% Mn, 0.02% Mo, 1.3% EDTA; Plant Products Company Ltd.). The plants were grown and watered daily in a greenhouse under the following conditions: 22°C/18°C (day/night) temperature, relative humidity of 20-35% and under natural light supplemented with incandescent lamps to maintain a 16-h photoperiod.

### *3.2.2 Treatments*

Drought treatments were applied to plants approximately three months after rooting. All pots were saturated with water and allowed to dry until a desired level of drought was achieved. The following treatments were applied: mild drought (≈ 25% soil water content, SWC), severe drought (≈ 12% SWC), severe drought followed by 30 minutes of recovery (rewatering to >40% SWC), severe drought followed by two days of recovery (rewatering to >40% SWC), and control (>40% SWC, well-watered) plants. Severe drought produced leaf wilting and some leaf necrosis but did not affect plant survival. Soil water content (SWC) was measured daily using time-domain reflectometry (TDR) (Topp *et al.*, 1980). The soil moisture content for mild and severe drought was selected based on a preliminary experiment (Appendix A). The aim was to produce stress levels, resulting in a reduction of stomatal conductance without the loss of leaf turgor (mild stress), and induce wilting (severe stress). This was achieved after five (mild) and seven (severe) days of treatment.

### *3.2.3 Soil water content*

An initial analysis of water retention capacity was obtained for the growing medium used in this study. Physiological measurements that are described below were obtained from four groups of plants exposed to different water regime treatments. The first group exposed to drought and recovery treatments, as described above, was assigned to stomatal conductance and water potential measurements. The second group of plants exposed to either severe stress or well-watered control was used for measuring stomatal aperture. The third group of well-watered control plants was taken for stomatal size and length measurements. The fourth group of well-watered control plants was used for the measurements of xylem vulnerability to cavitation.

#### *3.2.3.1 Retention curve for soil water potential*

To characterize the soil water availability to plants, retention curves were developed for Metro Mix® 290. To determine the soil volumetric water content at the wetter points of the curve, the pressure plate and tension table methods were used; and for the more negative points of the curve, the freezing-point depression method was used. The soil volumetric water content was determined using the tension table method (Reynolds & Topp, 2008). A 15-cm high column was filled with Metro Mix® 290 on top of a circular tank containing a saturated layer of water (tension table), to a dry bulk density of 0.2 g $cm^{-3}$, and horizontal TDR probes were inserted into the soil at 5, 7, 9, 10 and 11 cm above the tension table. The filter membrane allowed the Metro Mix® 290 to stay in hydraulic contact with a water reservoir even under negative pressure. Water potential was then determined by measuring the distance between the TDR probes and the water level in the reservoir. The Metro Mix® 290 was initially saturated and allowed to drain by lowering the water reservoir below the tension table. Soil water content was monitored with the TDR probes. Water content usually stabilized after three to five days of drainage and it was assumed to be at the water potential calculated from the distance between the water level in the reservoir and the TDR probe.

The second, freezing-point depression method, imitates field conditions in undisturbed soil and a soil-air interface as an upper boundary condition. This method is based on measuring the dielectric constant of the soil, which is a function of the volumetric liquid water constant. Therefore, a TDR was used to measure the unfrozen water content, and measurements of temperature and differences in radial temperature. The freezing point depression of pure water was also measured with the thermocouples to determine appropriate temperature offsets. Suzuki (2004), gives the following relationship between soil water potential and freezing point depression:

$$\Psi = 1.23\ T - \pi \qquad [3]$$

where T (in °C) is the freezing point depression, and $\pi$ is the osmotic potential. Since distilled water was used, the osmotic potential was assumed to be negligible. Equation [3] was derived using the Clapeyron equation:

$$P/\rho = T\ (L/273.15) \qquad [4]$$

where P is the matric potential, ρ is the density of water, T is temperature (in °C) and L is the latent heat of freezing. This gives a conversion factor between temperature and matric potential of 1.22 MP/K and is based on an assumption of similitude between freezing/thawing and drying/wetting (Johnson *et al.*, 1995).

### *3.2.4 Experimental design and measurements*

#### *3.2.4.1 Stomatal conductance and water potentials*

This experiment used a completely randomized block design with two treatment factors (drought and clone) with five drought treatment levels and seven poplar clones. Each drought treatment was applied to three trees per clone in two blocks. Blocking was in time and the experiment was carried out for two weeks. On each day, a single treatment *versus* its control was measured on the seven poplar clones.

At the end of the water withholding period, stomatal conductance and leaf and stem water potentials were measured. The measurements were taken for all drought treatments (drought, drought recovery and control) on the leaf corresponding to leaf plastochron index (LPI) 8. This index was used as a morphological measure of the leaf age (Larson & Isebrands, 1971;Ceulemans *et al.*, 1988); where LPI=0 was an unrolled leaf to about midpoint. Stomatal conductance measurements were carried out from 11.00 to 13.00 h using a LI-COR 6400 portable open gas exchange system (LI-COR Inc, Lincoln, NE, USA). The plants were exposed for five minutes prior to measurements to red/blue light (6400-02B LED Light Source) of approximately 1200 μmol $m^{-2}$ $s^{-1}$. Leaf temperature in the chamber was controlled (18 to 20°C) using a Peltier cooling system. Stomatal conductance was measured three times on each exposed leaf and the mean of the three measurements was used for the analysis.

After performing stomatal conductance measurements, water potentials of leaves ($\Psi_{leaf}$) and stems ($\Psi_{stem}$) were measured using a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) (Scholander *et al.*, 1965). For the measurements of leaf water potentials, fully-elongated leaves at LPI 9 were taken. For stem water potential measurements, LPI 10 was selected from each plant and was enclosed for 18 h in plastic bags and wrapped in aluminum foil to minimize transpiration water loss and create water potential equilibrium between the non-transpiring leaf and the stem.

#### *3.2.4.2 Stomatal aperture*

The stomatal aperture experiment was of a completely randomized design with two drought treatments (severe drought and well-watered control) and seven poplar clones with three replicate trees per treatment combination. A single leaf was selected on each tree with three measurements taken per leaf. Water was withheld for one week prior to the examination of stomatal aperture.
Poplar leaves have wide interspecific as well as interclonal variation in stomatal characteristics (*ie.*, density, size and structure) (Pallardy & Kozlowski, 1979; Ceulemans *et al.*, 1984; Ferris *et al.*, 2002; Al Afas *et al.*, 2006). Some poplars are amphistomatous (on both sides of the leaf) (Reich, 1984); with the majority of

stomata being on the abaxial surface (Blake *et al.*, 1984; Pearce *et al*; 2006), accounting for most of the water exchange in the plant. In this study, only abaxial stomata were examined. Stomatal aperture was measured in randomly selected abaxial leaf discs from the eighth leaf (LPI 8). Leaves were exposed for five minutes prior to measurement to LED light (LED ARRAY WHITE 1.25 x 1.05 SMD) up to 1200 μmol $m^{-2}$ $s^{-1}$. The leaf discs (1.16$mm^2$), selected from the leaf tip between the midrib and the leaf margin, were removed and immediately placed under a confocal electronic microscope (CEM) (Carl Zeiss, Inc., Oberkochen, Germany) equipped with a camera. The *in vivo* images were then digitized in three random fields of view where stomatal aperture was recorded.

### *3.2.4.3 Leaf stomatal density and size*

Leaf stomatal density and length were examined using a completely randomized design. A total of six well-watered trees per clone were used to examine stomatal density and length, in seven clones.

To determine the density and length of the stomata, the abaxial epidermis of LPI 8 was carefully smeared with nail varnish in the mid-area between the central vein and the leaf edge, and dried for approximately 5 minutes. Four thin films per leaf (approximately 5mm x 15 mm each) were peeled off from the leaf surface and mounted on a glass slide. The films were viewed and photographed using the electron photomicroscope system (Zeiss Axioskop 40 - Carl Zeiss, Inc., Oberkochen, Germany). Stomatal length was measured between the junctions of the guard cells (Malone *et al.*, 1993). For each leaf, four fields-of-view per slide were averaged for each slide to estimate density and length.

### *3.2.4.4 Xylem vulnerability to cavitation*

A total of six to eight well-watered trees per clone were used to evaluate clone differences in xylem vulnerability to cavitation in seven poplar clones in a completely randomized design experiment.

The vulnerability of xylem to embolism was determined in three-month-old rooted cuttings. Hydraulic vulnerability curves were constructed for six to eight

stems, each from a separate tree of the same clone, using the Cavitron technique (Cochard, 2002). Embolisms were measured as the hydraulic conductivity ($k_h$) versus the xylem water potential ($\Psi_{stem}$) of stem segments exposed to negative centrifugal forces (Sperry *et al.*, 1988; Cochard, 2002, Cochard *et al.*, 2005).

Plants were brought to the laboratory from the greenhouse and 50 cm long stem segments were cut underwater from each plant just below the pair of leaves corresponding to the apex of the plants. These stem segments were kept at 4°C in plastic bags with wet paper towel under dark conditions until measurements of hydraulic conductivity were performed within 24 hours after harvest. Just before each measurement, both ends were re-cut under water. The stem sections of 4 to 6 mm in diameter and 28-cm in length were then placed in the centrifuge. Although the vessel length and distribution was not determined, previous studies have found that the average vessel element length in mature poplar trees is in the range of 0.58 – 0.67 mm (Balatinecz *et al.*, 2001).

In the Cavitron technique, stem conductivity, $k_h$, can be measured while the stem segment spins in the Cavitron (Cochard *et al.*, 2006). Maximum conductivity, $k_{max}$, was measured at a low spin rate < 1500 RPM which corresponded to a xylem fluid pressure (minus tension) of >-0.5 MPa. As RPM is increased in steps of 100-RPM multiples, the tension increases causing $k_h$ to fall due to cavitation events. The percentage loss of hydraulic conductivity (PLC) was plotted against the xylem water potential (MPa). The PLC was then calculated using the following equation:

$$PLC = 100(k_{max} - k_h)/k_{max} \quad [5]$$

where $k_{max}$ is the maximum conductivity and $k_h$ is the hydraulic conductivity at a given tension.

In order to determine the relationship between xylem water potential ($\Psi_{stem}$) and cavitation, the vulnerability curve data were fitted based on a sigmoidal equation (Pammenter and Van der Willligen, 1998) and modified following Ratrowsky (1990):

$$PLC = 100 /(1+ \exp[b-\Psi_{stem}]/a) \quad [6]$$

where the value of 100 is the maximum loss in conductivity, $\Psi_{stem}$ is the xylem water potential, coefficient *a* is a shape parameter that relates to the range of water potentials over which conductivity is lost; and *b* is a position parameter of the curve and represents the $\Psi_{stem}$ at which a 50% loss ($P_{50}$) in conductivity occurs.

### *3.2.5 Statistical analysis*

Analysis of variance (ANOVA) was performed using the *Proc Mixed* procedure of SAS (9.1) (SAS Institute, Cary NC US, 2002). The assumption of normality was assessed using the Proc UNIVARIATE function with the Kolmogorov-Smirnov test for normality. Drought treatment effects were analyzed using one way analysis of variance for each clone with block and block interaction terms considered random. A Kenward-Roger option was used to calculate denominator degrees of freedom. Least squared means were separated using the pdiff option, and a significant level of $\alpha$=0.05 was used to determine significances between treatments. Stomatal density and size data were log-transformed to satisfy assumptions of normality and homogeneity. Differences between clones for stomatal density and size, and loss of conductivity were analyzed using one way analysis of variance. Linear models and ANOVA tables for each analysis are shown in Appendix B.

#### *3.2.5.1 Vulnerability curves*

Vulnerability curves were analyzed using a generalized nonlinear regression model with heterogeneous and autocorrelated error terms (Pinheiro *et al.*, 2007), to determine whether differences in coefficients between clones were statistically significant. Heterogeneous error terms were needed to compensate for the higher variability in the central part than in the extremes of the curves (Pinheiro & Bates, 2000). Autocorrelated errors were included in the model to take into account the fact that the same stem was being measured over a range of tensions, so measurements were likely to be partially dependent (see Pinheiro & Bates, 2000). Generalized least squares were used to fit the logistic curve

(equation 6) to the vulnerability curve data and compare the model for the different poplar clones (Pammenter & Van der Willigen, 1998). Parameters *a* and *b* (equation 6) were estimated using the extended non-linear regression function `gnls` (generalized nonlinear least squares) available in the library nlme (nonlinear mixed effect model) in R software (Pinheiro *et al.*, 2007). A full (maximal) model, with independent coefficients of *a* and *b* for each sample, was initially fitted to the whole set of samples. Sequential model simplification was performed to find the minimal adequate model, *i.e.* the model with the minimum number of coefficients that adequately fit the different poplars. Hybrids and balsam poplar were considered significantly different for the *a* parameter (slope of the curve) when it could not be fitted adequately with a single coefficient. Differences between poplar clones for parameter *b* ($P_{50}$), upper ($P_{10}$) and lower ($P_{90}$) vulnerability threshold were analyzed using one way anlysis of variance.

### *3.3 Results*

#### *3.3.1 Soil water potential*

Soil moisture content from TDR measurements were used to calculate soil water potential ($\Psi_{soil}$) for each treatment based on the soil retention curve constructed for the Metro Mix® 290 media (Appendix C). There were statistical differences for $\Psi_{soil}$ values under different treatments. Control and the two recovery treatments were statistically different from severe and mild treatment for all the poplar clones. Comparison of recoveries and controls were only different for Northwest and Berlin. Where soil water potential values for the Northwest clone in recovery treatment after 30 minutes of re-watering was statistically different (more negative) than control ($p=0.01$). Soil water potential values for recovery after 2 days in the Berlin clone was more negative than control plants ($p=0.03$). Overall, $\Psi_{soil}$ values for severe drought were higher (more negative) than mild drought. $\Psi_{soil}$ ranged between -0.001 to -0.002 MPa for control plants, -0.018 to -0.09 MPa for mild stress, -0.54 to -2.27 MPa for severe stress, and -0.002 to -0.005 MPa for both stress recovery treatments (Fig. 3.1). Nevertheless

soil water potentials in drought conditions were more negative than leaf water potential values (Figs. 3.1 and 3.3).

### *3.3.2 Stomatal conductance ($g_s$), water potentials ($\Psi_{leaf}$) and stomatal aperture*

Stomatal conductance in Assiniboine was relatively high in well-watered plants and increased in response to mild drought (p=0.01). Severe drought induced decreases in $g_s$ (p=0.001), and no recovery after 30 minutes of re-watering was observed. However, after one day of re-watering, $g_s$ values returned to the control level (Fig. 3-2A). In P38P38, decreases of $g_s$ by about 40% were observed in mild (p=0.0009) and severe (p=0.004) drought, and drought recovery treatments (p=0.0001 for the 30-min. and p<0.0001 for the 2-day recovery) compared with well-watered control plants (Fig. 3-2B). The Northwest clone decreased stomatal conductance only under severe drought conditions (p=0.01), and $g_s$ levels were gradually recovering after 30 minutes and 2 days of re-watering (Fig. 3-2C). Stomatal conductance declined in Okanese and Berlin under severe drought (p=0.004 and p=0.02, respectively); $g_s$ only recovered after two days of re-watering (Figs. 3-2D and E). Conversely, drought treatments did not have an effect on $g_s$ in balsam poplar; however, lower values of $g_s$ in plants after 30 minutes of re-watering, compared with control were observed in this poplar (p=0.01) (Fig. 3-2F). Decreases in $g_s$ were observed in Walker for severe drought (p=0.04) and drought recovery after 30 minutes (p=0.02) (Fig. 3-2G).

Overall, a decrease in leaf water potential occurred in all the clones under severe drought, with the exception of Walker which had higher $\Psi_{leaf}$ values under severe drought stress compared with well-watered control plants (Fig. 3-3G). Decreases of more than twice in $\Psi_{leaf}$ were observed in Northwest (p=0.0006), Berlin (p=0.0001) and balsam poplar (p<0.0001) compared with controls (Fig. 3-3C, E, F). A rapid recovery capacity was also observed in all the poplar clones, with $\Psi_{leaf}$ values similar to control plants after 30 minutes of re-watering. Stomatal conductance and leaf water potentials after 30 minutes of re-watering were not measured in the Berlin clone due extensive leaf damage in plants exposed to severe drought.

Stomatal apertures were measured in the leaves of well-watered and drought-stressed plants collected during the midday (Fig. 3-4). In well-watered plants, stomatal aperture varied from about 2.8 μm in Walker to about 6.3 μm in Northwest and balsam poplars (Fig. 3-4). Stomatal aperture significantly decreased in response to severe drought stress in Assiniboine, P38P38, and Okanese clones, while no differences between well-watered and stressed plants were recorded for Northwest. The stomatal aperture was greater in stressed compared with well-watered plants in balsam poplar and Berlin clones (Fig. 3-4).

There were significant clone differences ($p<0.001$) for leaf stomatal density and Northwest and Okanese clones had the lowest stomatal density with about 100 and 120 stomata per $mm^2$, respectively (Fig. 3-5A). The number of stomata per $mm^2$ of a leaf ranged from about 130 to 170 in Berlin, balsam poplar and Walker clones (Fig. 3-5A). The highest leaf stomatal density was found in P38P38 and Assiniboine clones with 195 and 220 and stomata per $mm^2$ respectively (Fig. 3-5A).

There were also significant differences in stomatal length between the clones, with Northwest and balsam poplar having the stomata that were almost twice as long as those present in the leaves of Berlin plants (Fig. 3-5B).

### *3.3.3 Xylem vulnerability to cavitation*

The vulnerability curves for Okanese, P38P38, Walker, balsam, Berlin and Northwest had an "s" shape and were successfully-fitted with a sigmoid function ($r^2>0.98$) (Fig. 3-6). However, the curves in Assiniboine showed an atypical double sigmoid shape and there was high variability between plants. Therefore, this clone was not included in this analysis.

The rate at which increases of tension induced cavitation, was significantly different between the clones ($p<0.001$). Based on the slope of the curve, the clones were grouped into the following categories: clones relatively high resistant to cavitation (Northwest, balsam poplar, and P38P38) for the clones that underwent xylem cavitation at lower water potentials; clones with relatively low resistance to cavitation (Berlin and Okanese) for the clones undergoing rapid cavitation at higher water potentials; and clones with

intermediate resistance to cavitation (Walker) (Fig. 3-6). Stem water potential ($\Psi_{stem}$) at which cavitation began was -1.25 MPa for Northwest and balsam poplar and -0.85 MPa for P38P38, respectively. For Walker, xylem cavitation started at -0.75 MPa $\Psi_{stem}$ (Fig. 3-6). However, the plants of this clone displayed a small native state embolism, below 10%. In Berlin and Okanese clones, cavitation onset occurred at -0.65 MPa $\Psi_{stem}$ (Fig. 3-6).

Quantitative analysis of vulnerability to drought-induced embolism revealed that Northwest and balsam poplar reached 10, 50 and 90% loss of conductivity at more negative $\Psi_{stem}$ values compared to the rest of the clones ($p<0.0001$) (Fig. 3-7). These poplars also exhibited lower slopes of the vulnerability curves (Fig. 3-6). Loss of conductivity at 10% ($P_{10}$) was reached first by the Walker clone ($\Psi_{stem}$ -0.4 MPa), followed by Okanese ($\Psi_{stem}$ -0.6 MPa) and Berlin ($\Psi_{stem}$ -0.84MPa) (Fig. 3-7). Stem water potential values at $P_{50}$ ranged from -1.0 MPa for the clone most vulnerable to cavitation (Okanese) to -1.6 MPa for the least vulnerable clone (Northwest). $P_{90}$ (90% loss of conductivity) was reached first by P38P38 ($\Psi_{stem}$ -1.42), followed by Okanese ($\Psi_{stem}$ -.1.45) and Berlin ($\Psi_{stem}$ -1.55) (Fig. 3-7).

Vulnerability curves were also constructed for the percentage loss of conductivity due to xylem embolism and midday stomatal conductance ($g_s$) as a function of stem water potential (MPa) along stems of rooted cuttings (Fig. 3-8). The lowest stomatal conductance reported in Fig. 3-8 under severe drought treatment occurred at $\Psi_{stem}$ values that varied between -0.6 MPa for Okanese, P38P38, Walker and balsam poplar, to -0.9 MPa for Northwest and Berlin. Under severe drought, Okanese plants significantly decreased $g_s$ and embolisms occurred (≈12% PLC) in the xylem at $\Psi_{stem}$ of -0.6 MPa (Fig. 3-8C). In Berlin and Assiniboine subjected to severe drought stress, occurrence of embolisms (20% and 40 PLC respectively) was observed at $\Psi_{stem}$ values of -0.9 and -0.6 MPa, respectively (Figs. 3-8D, G). Walker showed embolism (12% PLC) under severe drought; however, rapid recovery was observed after rewatering, (Fig. 3-8F).In balsam poplar, severe drought corresponded to -0.72 MPa $\Psi_{stem}$, and no embolisms were observed. Northwest showed differences in $g_s$ between severe drought and control, nonetheless no embolisms were observed up to -0.84 MPa $\Psi_{stem}$ (Fig. 3-8B). P38P38 closed the stomata under drought treatment

decreasing the stomatal conductance under both mild and severe stress. However, for $\Psi_{stem}$ the values for both recovery times (30 minutes and 2 days) were higher (less negative) than the control values.

### *3.4 Discussion*

In the present study, soil water potential in well-watered control plants was maintained between -0.001 and -0.002 MPa. Poplars are sensitive to the soil moisture availability and, in general, require soil water potentials ($\Psi_{soil}$) above -0.025 MPa during their growth period (Schock *et al.*, 2002). Severe drought stress treatment lowered $\Psi_{soil}$ to between -0.54 and -2.27 MPa. This variability in $\Psi_{soil}$ likely reflected differences in water use by the different plants. In addition, soil water potential was calculated based on soil water content, measured using the TDR with an accuracy of ±2% and precision ±1% (Hillel, 1998). The possible source of inaccuracy in TDR measurements may be due to air gaps, since soil tends to shrink upon drying (Topp *et al.*, 1980; Hillel, 1998), or to external fluctuations in temperature (Evett *et al.*, 2005). Therefore, soil water potential calculations based on the TDR system should be interpreted with caution.

Northwest and balsam poplar had larger stomatal size and aperture compared to the other five clones. Stomatal size has been linked to gas exchange efficiency and maintenance of leaf water balance. A similar stomata aperture pattern was also reported by Silim *et al.* (2009) in the Northwest clone. Stomatal aperture is closely related to the stomatal size (Xu & Zhou, 2008), which may indicate better $CO_2$ diffusion into the leaf (Pakhurst, 1994), high stomatal conductance (Maherali *et al.*, 2002), and greater capacity for turgor maintenance (Cutler *et al.*, 1977). On the other hand, the present results do not show a direct association between stomatal density and stomatal conductance as reported by Pearce *et al.*, (2006) for other poplars. In poplar clones with greater stomatal aperture under severe stress, greater leaf necrosis was observed. This was particularly apparent in Berlin and balsam poplar. Similarly, Northwest plants kept the stomata open when subjected to severe stress, resulting in an almost two-fold decrease in leaf water potential, and leaf chlorosis and shedding of older leaves was observed. Previous studies in *Populus* have found that under drought

conditions a reduction in leaf area by leaf senescence and abscission provides an additional mechanism to reduce transpirational demand (Rood *et al.*, 2000; Amlin & Rood, 2003), and increase reallocation of nutrients in younger leaves (Chaves *et al.*, 2003; Bacelar *et al.*, 2007).

Balsam poplar plants maintained high stomatal conductance under drought stress conditions and showed relatively high resistance to xylem cavitation. It is expected that plants of the clones that maintain open stomata under drought conditions would also have higher rates of transpiration and lose more water than other clones; hence the conducting system is also expected to be less vulnerable to embolism (Jones & Sutherland, 1991).

Although Walker plants also maintained open stomata under severe drought, they likely avoided water loss since they maintained relatively high $\Psi_{leaf}$. The Walker clone showed initial cavitation in vessels, indicating that it had undergone some water stress in the greenhouse. Conversely, Assiniboine, Okanese and P38P38 responded to severe drought stress by closing the stomata and decreasing stomatal conductance (Table 3-1). Although decreases in leaf water potential were observed, leaf necrosis did not become visible in these clones when exposed to the severe stress treatment. Stomatal closure in response to soil water scarcity is a common response, and the extent and speed of this response differs among poplars (Hukin *et al.*, 2005; Silim *et al.*, 2009). Stomatal control appeared to be important in maintaining $\Psi_{leaf}$ at a value of stem water potential that is above the threshold for "runaway cavitation" (Tyree & Sperry, 1989). However stomatal closure increases plant survival in the short term, and leads to a decrease in photosynthesis and therefore, productivity (Jones & Sutherland, 1991). Stomatal response observed in this study, for Assiniboine, P38P38 and Okanese clones, is consistent with the studies of Salleo *et al.*, (2001) and Broddrib & Holbrook (2003) studies, in which leaves of different tree species operated close to the point of cavitation and stomatal closure during water stress and was closely tied to the prevention of cavitation in the xylem.

Although poplars are considered to be among the tree species most sensitive to cavitation (Tyree *et al.*, 1992; Hacke & Sauter, 1995; Hacke *et al.*, 2001; Cochard *et al.*, 2007) and require large amounts of water (Tschaplinski *et al.*, 1994; Kim *et al.*, 2008), we found two strategies in the different poplar clones to soil water depletion: cavitation avoidance and tolerance. The avoidance

strategy relies on stomatal closure to maintain high $\Psi_{stem}$ values since the xylem is relatively vulnerable to cavitation. This strategy characterizes the responses of P38P38 and Okanese clones to drought stress (Figs. 3-4, 3-8). The second strategy allows the plants to maintain open stomata and photosynthesize longer in response to drought stress and therefore, it can be considered a drought tolerance strategy. This strategy was present in poplar clones which showed greater xylem resistance to cavitation (Northwest, balsam poplar and Walker) (Figs. 3-4, 3-8; Table 3-1). A remarkable characteristic of Walker was that its xylem was relatively resistant to cavitation and the plants kept their stomata open under drought conditions without a large decline in leaf water potential. This may represent a decoupling mechanism, by maintenance of high root hydraulic conductivity (not reported here) or by osmotic adjustment (Tschaplinski & Blake, 1989; Tschaplinski & Tuskan, 1994). Plants need to maintain a small margin of safety in order to prevent cavitation and reductions in water transport and consequently maximum gas exchange. Therefore, these mechanisms of avoidance and tolerance to cavitation are also directly related to drought resistance (Maherali *et al.*, 2004). Despite the fact that the xylem in plants of the Berlin clone was relatively sensitive to cavitation, poor stomatal control suggests that the plants of this clone may be susceptible to drought stress conditions. Therefore, the efficiency of stomatal control of water loss is an important factor which must be taken into account when determining the probability of xylem cavitation in stressed plants (Sperry *et al.*, 1993; Ennajeh *et al.*, 2008).

Xylem structure is an important component of resistance to cavitation. Wide xylem vessels develop cavitation at higher water potentials compared with narrow and short vessels (Tyree & Dixon, 1986; Tyree & Zimmerman, 2002). Therefore, the differences in slopes of the vulnerability curves found in this study among the seven clones suggest possible differences in xylem structure between clones. A steeper slope of the vulnerability curve implies more cavitated vessels per unit of change in $\Psi_{stem}$ (Pammenter & Vander Willigen, 1998). The two-sigmoid shape of the vulnerability curve for Assiniboine is difficult to explain but might suggest that this hybrid has vessels with larger diameters that cavitated first before they reached the first plateau when small vessels started to cavitate. This could be explained based on the pit area hypothesis (Hacke *et al.*, 2006), which postulates that the higher pit membrane area per vessel, the greater the

maximum pit membrane pore and hence, the lower the air seeding threshold of cavitation to extend between vessels. Therefore, the larger the pore size, the more vulnerable vessels are to cavitation by the capillary forces (Wheeler *et al.*, 2005; Hacke *et al.*, 2006). In addition, a previous study by Silim *et al.* (2009) classified this clones as drought-sensitive.

Since xylem cavitation reduces xylem hydraulic conductivity, the water potential difference between soil and leaf must increase if the plant maintains constant water flow and gas exchange rate (Sperry *et al.*, 1993). Hence cavitation resistance (avoidance) may be advantageous to maintain cell turgor and growth. Similarly, avoidance mechanisms can be effective in keeping stem and leaf water potential within a limited range. The timed response of the stomata to reduced hydraulic conductivity was probably the main factor associated with preventing extensive loss of vessel conductivity due to induced cavitation. However, in the present study, even severe drought stress conditions did not produce very low xylem tensions in the studied plants.

Moreover, recovery responses after severe drought were also observed which resulted in similar or even higher values of $\Psi_{stem}$, $\Psi_{leaf}$ and $g_s$, compared with those in the control plants. Recovery mechanisms can be associated with the ability of some plants to repair embolism by refilling the vessels and improving water uptake (Canny, 1997; Tyree *et al.*, 1999; Hacke & Sperry, 2003; Salleo *et al.*, 2004, Maherali *et al.*, 2004). Although the exact processes contributing to embolism repair are not fully understood, aquaporins have been implicated in this process (Yamada *et al.*, 1997). It has been suggested that the aquaporins located in the radial membrane of the vessel-associated cells, xylem parenchyma cells and contact cells help fill up the embolized vessels with water (De Boer & Volkov, 2003; Galmés *et al.*, 2007; Kaldenhoff *et al.*, 2008). Leaf physiology is closely linked to hydraulic architecture but the mechanism by which stomata, and thus cavitation events are regulated, remains to be elucidated.

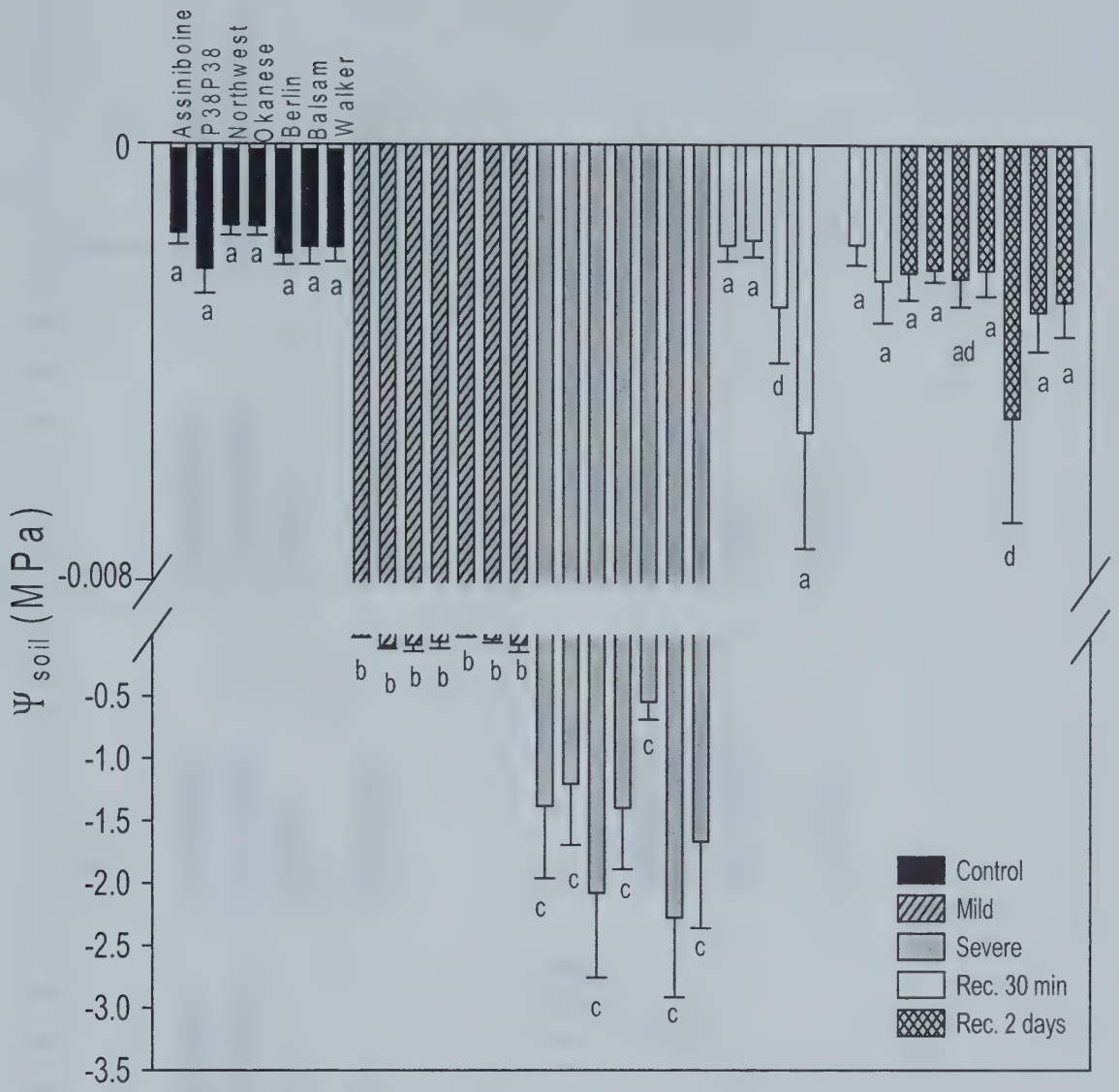



**Figure 3-1.** Soil water potential ($\Psi_{soil}$) (means +SE, n=6.) in control plants, mild and severe stress, and in plants rewatered for 30 minutes and 2 days. The order of the clones for each treatment is as described on the top left for the control. Clones with different letters are significantly different between treatments for each clone at $p \leq 0.05$.

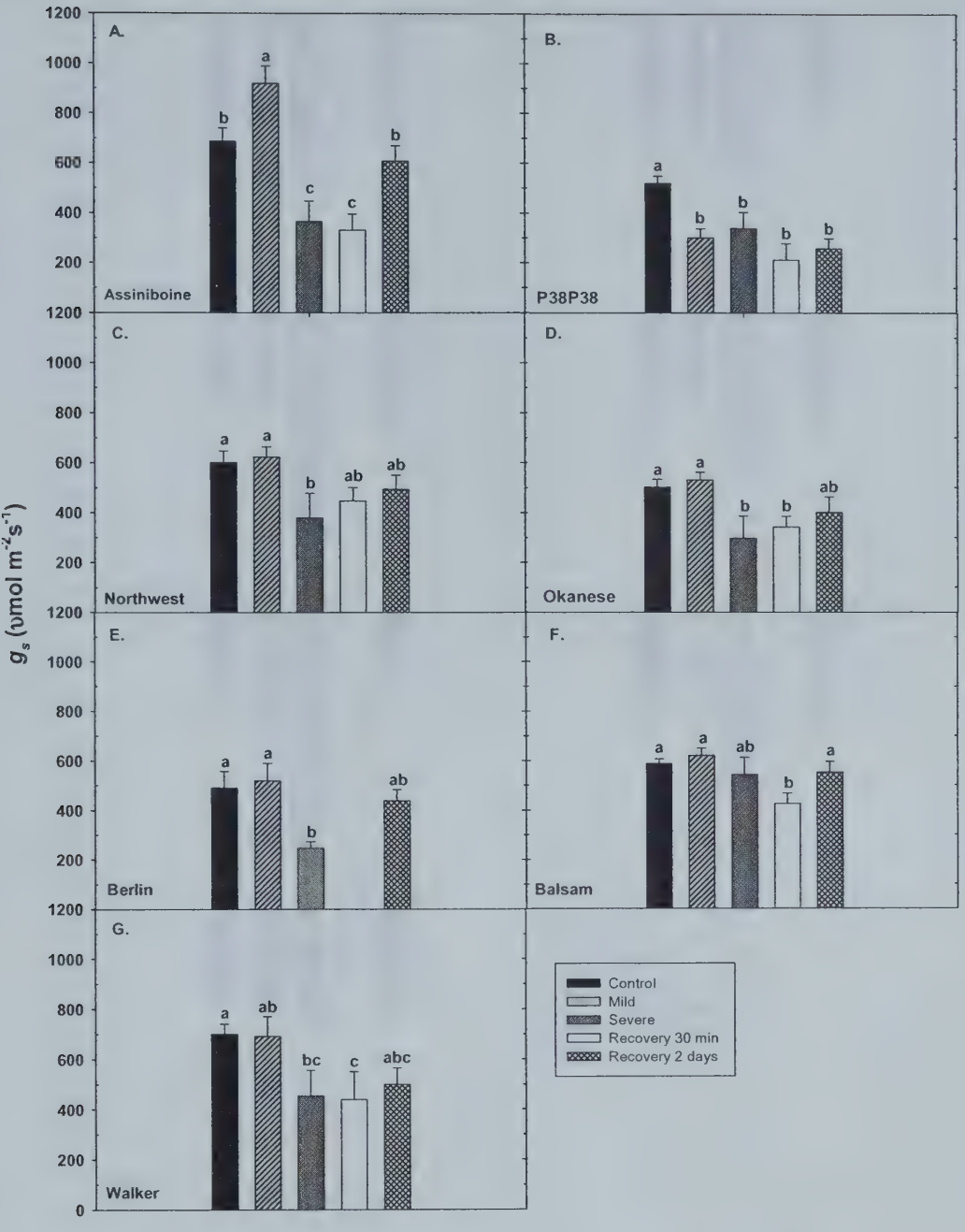



**Figure 3-2**. Stomatal conductance ($g_s$) (means + SE, n=6) of poplar clones subjected to mild drought stress, severe drought stress, 30-min stress recovery, and 2-day stress recovery. Different letters denote statistically difference between treatments for each clone.

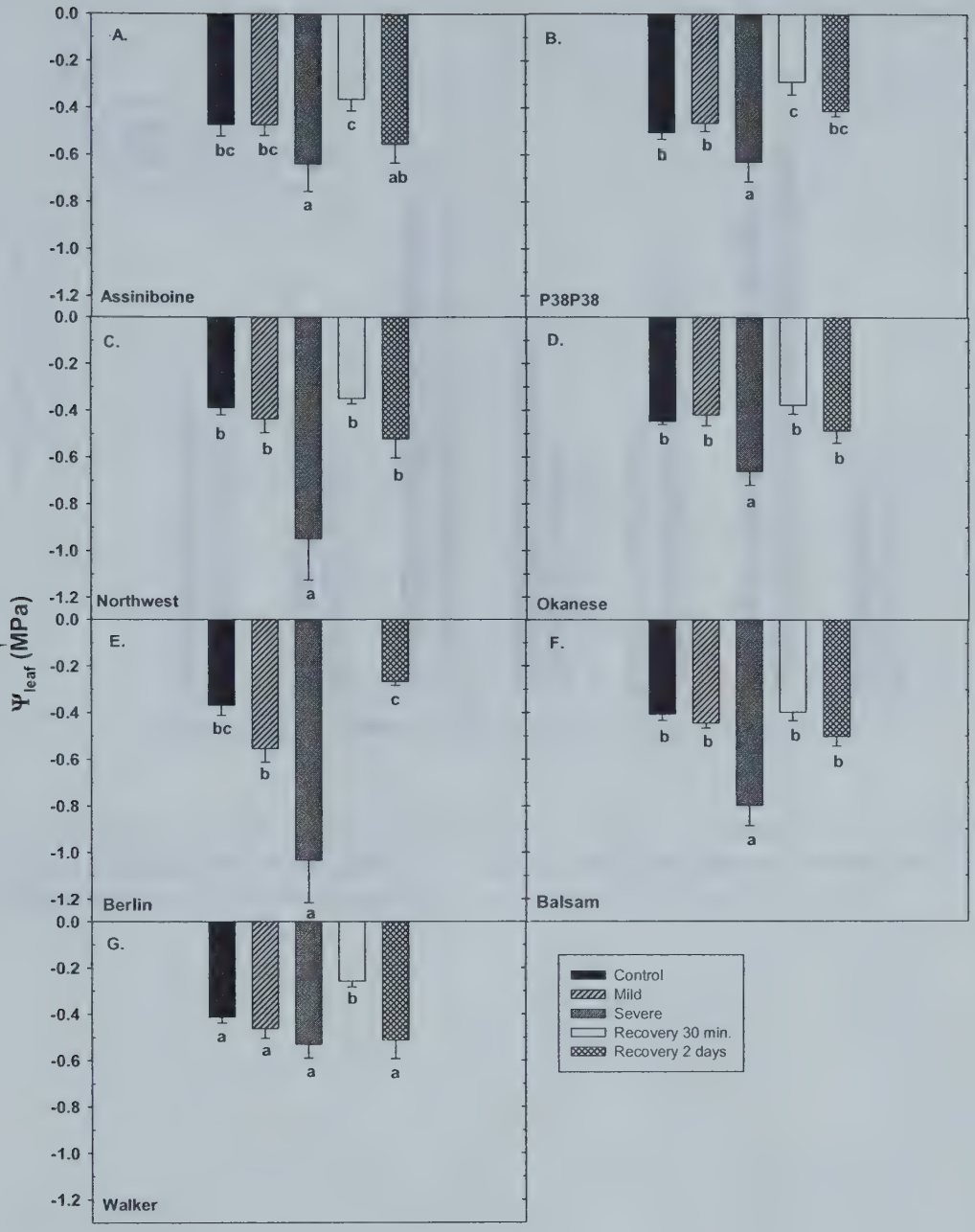



**Figure 3-3**. Leaf water potential ($\Psi_{leaf}$) (means + SE, n=6) of poplar clones subjected to mild drought stress, severe drought stress, 30-min stress recovery, and 2-day stress recovery. Different letters denote statistically difference between treatments for each clone.

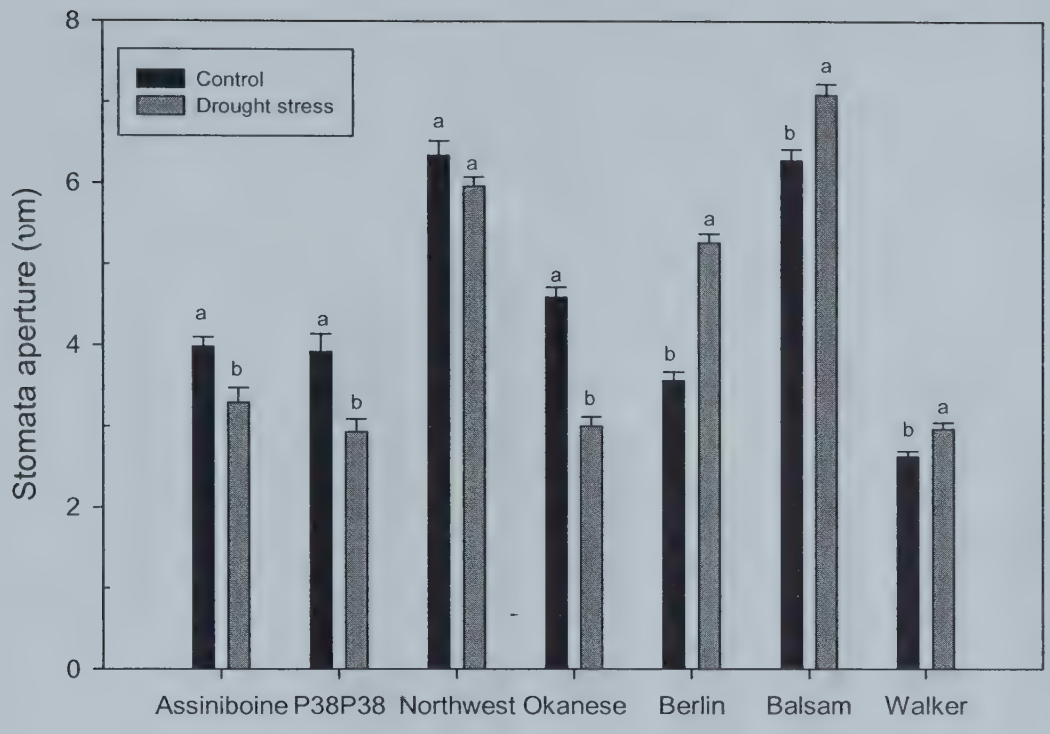



**Figure 3-4**. Stomatal aperture (means + SE, n=3) in well-watered control plants and plants subjected to severe drought stress. Clones with different letters are significantly different between the treatment and control for each clone at $p \leq 0.05$.

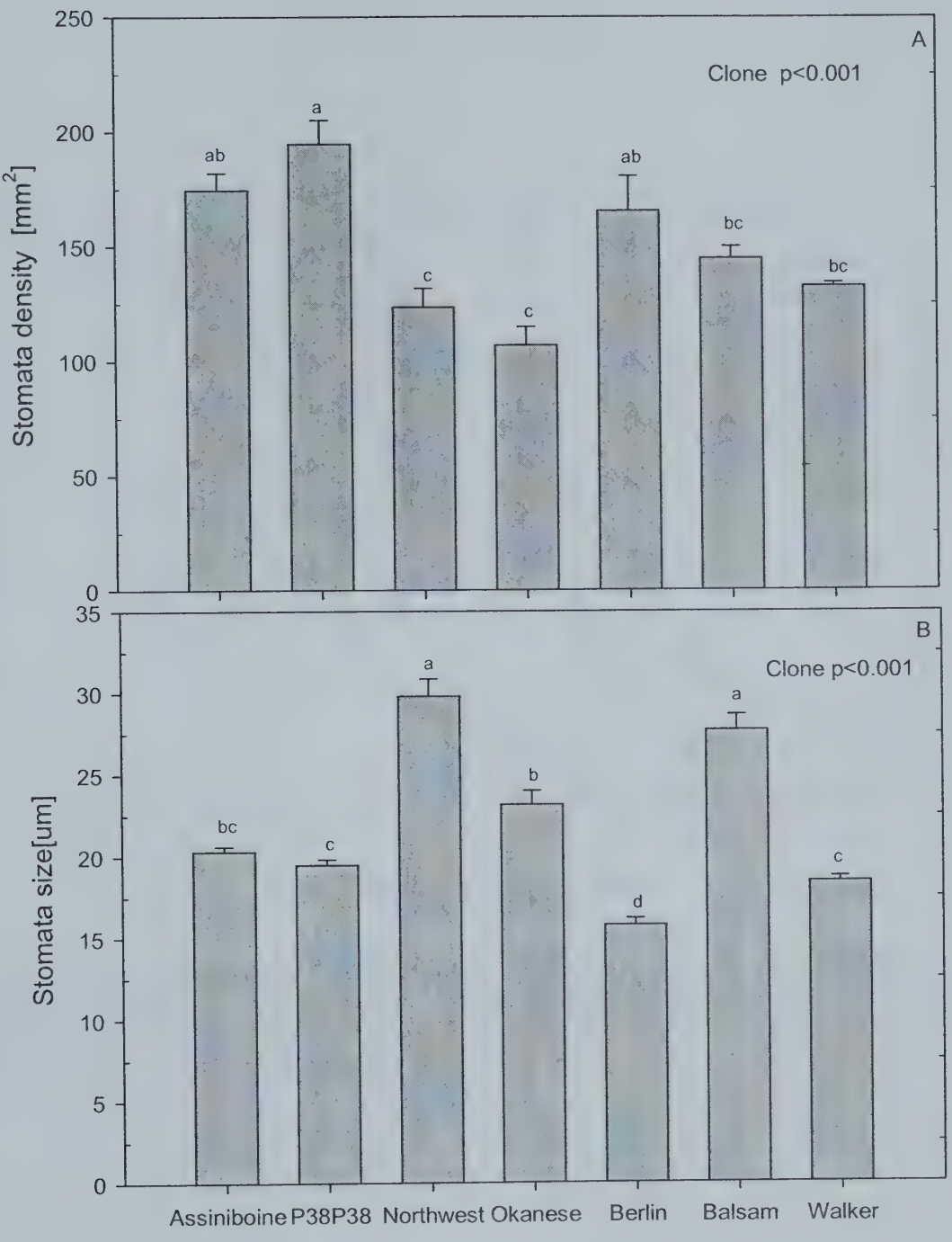



**Figure 3-5.** Abaxial stomatal density (A) and length (B) (means + SE, n=6) in seven poplar clones. Bars with different letters are significantly different.

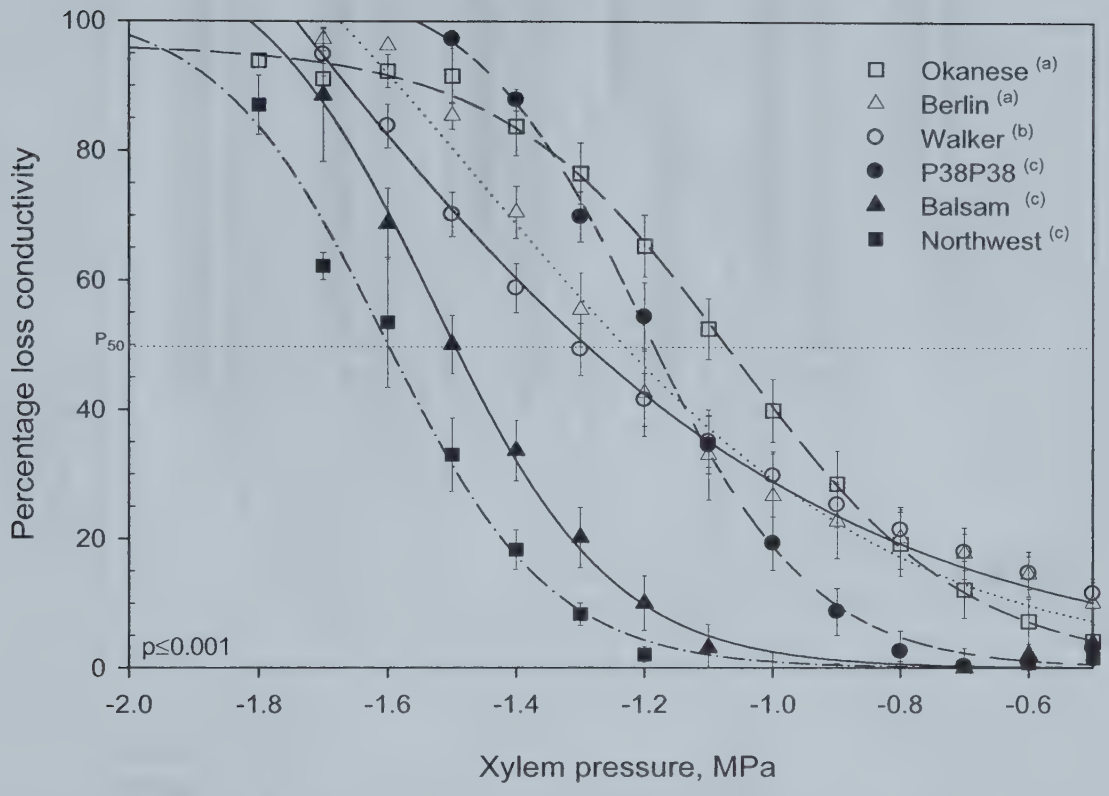



**Figure 3-6.** Xylem vulnerability curves of stem xylem in different poplar clones (means ± SE n=6-9). Curves show the percentage loss of conductivity (PLC) with decreasing xylem pressure. Lines are best fit sigmoidal curves. Horizontal dotted line shows $P_{50}$. Clones with different letters are significantly different in slope.

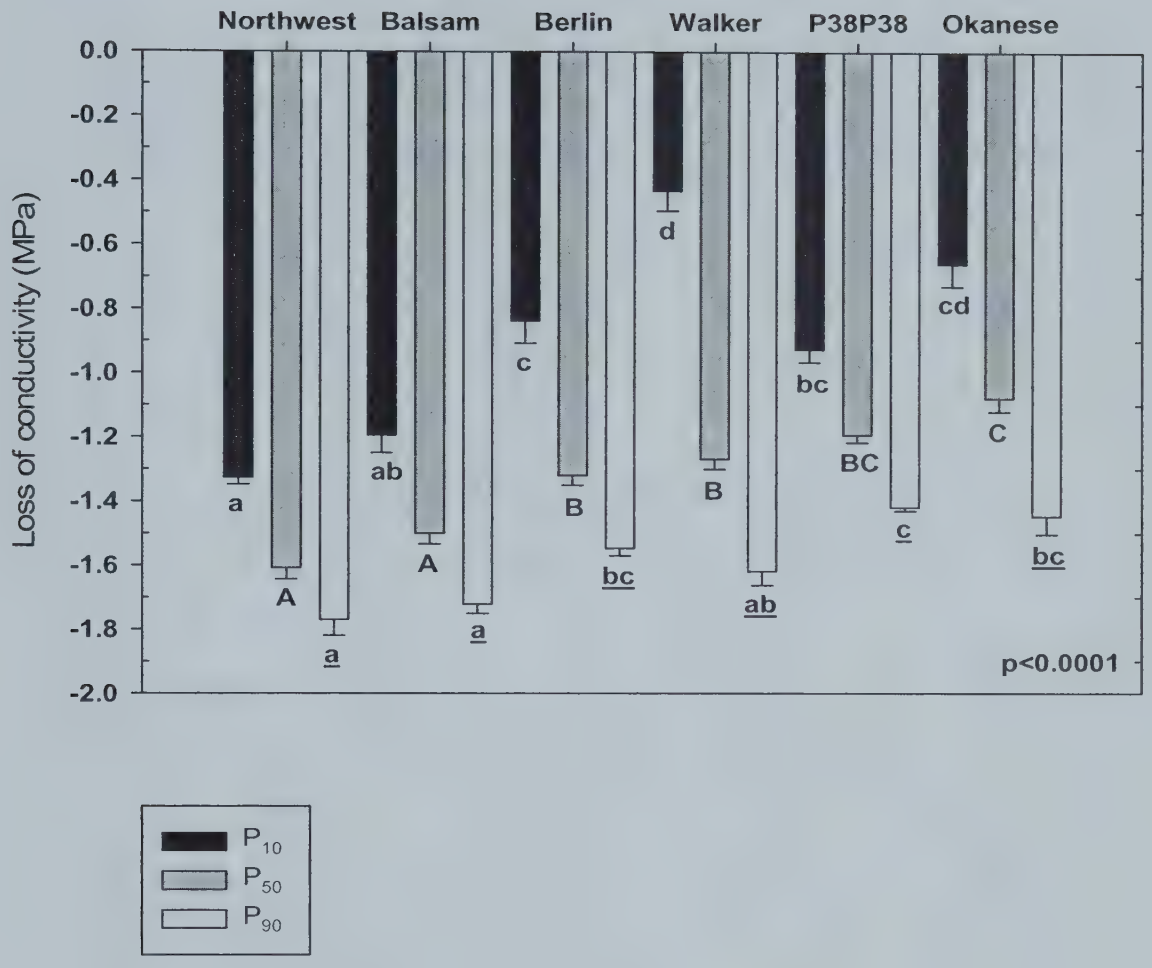



**Figure 3-7.** Parameter *b* (50% loss of conductivity, $P_{50}$), and the upper and lower vulnerability thresholds (10% and 90% loss of conductivity, $P_{10}$ and $P_{90}$) respectively, in different poplar clones. Means (+SE, n=6-9) with different letters are significantly different between clones.

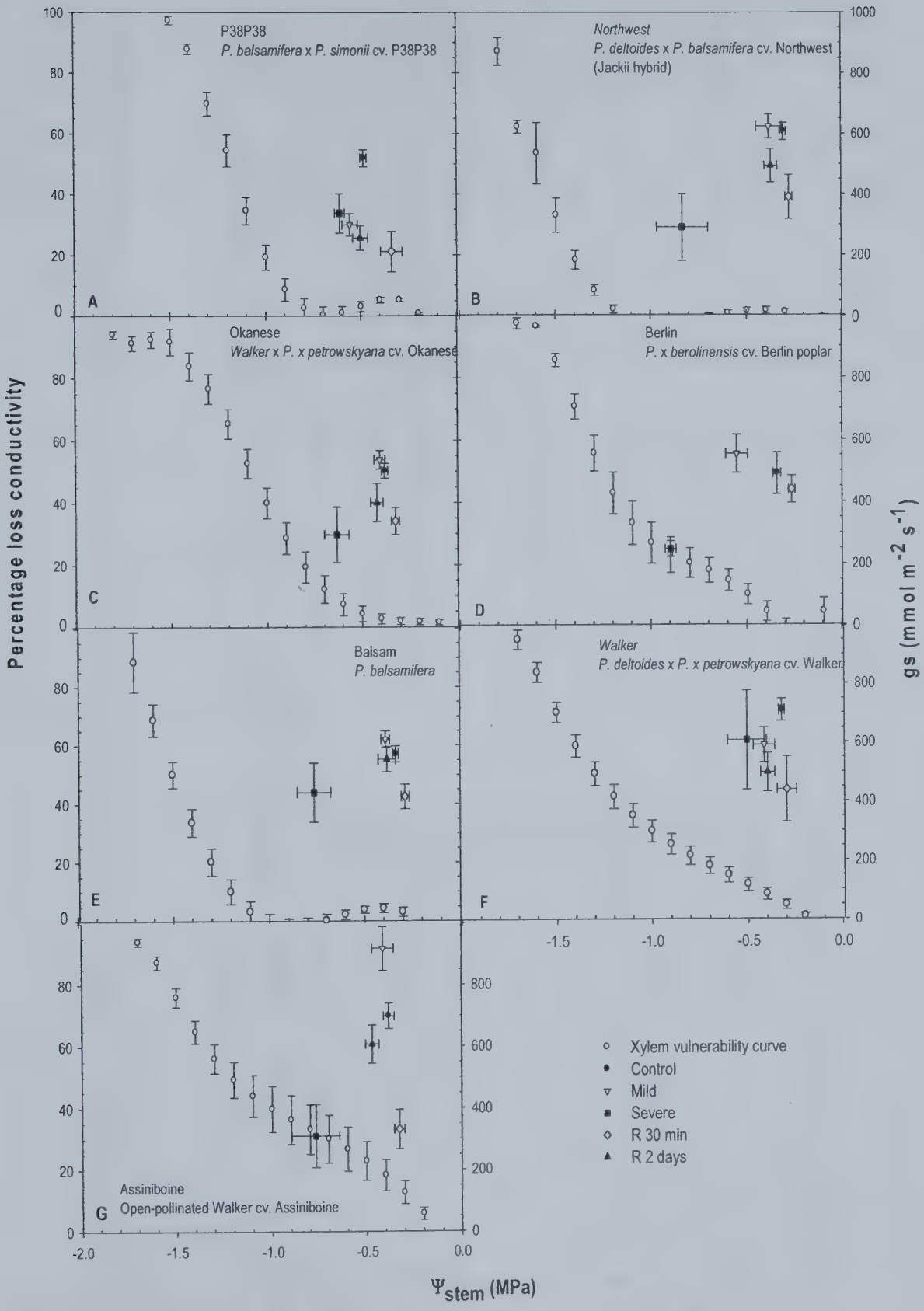



**Figure 3-8.** Vulnerability curves for the percentage loss of conductivity due to xylem embolism and midday stomatal conductance ($g_s$) as a function of stem water potential (MPa) along main stems of rooted cuttings for seven (A-G) poplar clones (±SE). Mean values of $g_s$ correspond to the different levels of drought and recoveries compared with the control (n=6).

**Table 3-1.** Summary of the physiological responses of the poplar clones under severe drought stress. Putative drought resistance classification was based on the combination of the physiological responses showed in this study. (↓) Decrease, (↑) increase or (↔) no changes compared with control plants.

| Poplar clone | Stomatal conductance* ($g_s$) | Leaf water potential* ($\Psi_{leaf}$) | Stem water potential* ($\Psi_{stem}$) | Stomatal aperture | Loss of conductivity $P_{10}$ | Loss of conductivity $P_{50}$ | Loss of conductivity $P_{90}$ | Vulnerability to xylem cavitation | Putative drought resistance |
|---|---|---|---|---|---|---|---|---|---|
| **Assiniboine** | 47% (↓) | 36% (↓) | 130% (↓) | Partially closed | | | | | Low |
| **P38P38** | 35% (↓) | 23% (↓) | 25% (↓) | Partially closed | -0.93 | -1.19 | -1.42 | Low | Moderate |
| **Northwest** | 37% (↓) | 140% (↓) | 189% (↓) | Greater stomatal aperture | -1.33 | -1.61 | -1.77 | Low | Moderate |
| **Okanese** | 41% (↓) | 47% (↓) | 43% (↓) | Partially closed | -0.66 | -1.08 | -1.45 | High | Low |
| **Berlin** | 50% (↓) | 170% (↓) | 110% (↓) | Greater stomatal aperture | -0.69 | -1.27 | -1.52 | High | Low |
| **Balsam** | (↔) | 95% (↓) | 140% (↓) | Greater stomatal aperture | -1.2 | -1.5 | -1.72 | Low | High |
| **Walker** | 35% (↓) | (↔) | 93% (↓) | Greater stomatal aperture | -0.46 | -1.25 | -1.63 | Moderate | Moderate |

* Estimated percentage of decrease or increase compared with control plants.

## References


Amlin NM, Rood SB (2003) Drought stress and recovery of riparian cottonwoods due to water table alteration along Willow Creek, Alberta. *Trees* **17**,351-358.

Bacelar EA, Moutinho-Pereira JM, Gonçalves BC, Ferreira HF, Correia CM (2007) Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. *Environ Exp Bot.* **60**, 183-192.

Balatinecz JJ, Kretschmann DE, LecLercq A (2001) Achievements in the utilization of poplarwood-guideposts for the future. *For Chron.* **77**(2), 265-269.

Blake TJ, Tschaplinski TJ, Eastham A (1984) Stomatal control of water use efficiency in poplar clones and hybrids. *Can J Bot* .**62**, 1344-1351.

Bond BJ, Kavanagh KL (1999) Stomatal behaviour of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential. *Tree Physiol.* **19**, 503-510.

Boyer JS (1982) Plant productivity and environment potential for increasing crop plant productivity, genotypic selection. *Science* **218**, 443-448.

Ceulemans R, Impens I, Steenackers V (1984) Stomatal and anatomical leaf characteristics of 10 *Populus* clones. *Can J Bot* . **62**, 513–518.

Ceulemans R, Impens I (1988). Genetic variation in aspects of leaf growth of *Populus* clones, using the leaf plastochron index. *Can J For Res.* **18**, 1069-1077.

Chaves MM, Maroco JP, Pereira JS (2003) Review: Understanding plant responses to drought – from genes to the whole plant. *Func Plant Biol.* **30**, 239-264.

Cochard H, Coll LI, Roux XL, Améglio R (2002) Unravelling the effects of plant hydraulics on stomatal closure during water stress in Walnut. *Plant Physiol.* **128**, 282-290.

Cochard H (2002) A technique for measuring xylem hydraulic conductance under high negative pressures. *Plant Cell Environ.* **25**, 815-819.

Cochard H, Damour G, Bodet C, Tharwat I, Poirier M, Améglio T (2005) Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiol Plant* **124**, 410-418.

Cochard H, Casella E, Mencuccini M (2007) Xylem vulnerability to cavitation varies among poplar and willow clones and correlates with yield. *Tree Physiol.* **27**, 1761-1767.

Cutler JM, Rains DW, Loomis RS (1977) The importance of cell size in the water relations of plants. *Physiol. Plant* **40**, 255-260.

De Boer Ah, Volkov V (2003) Logistic of water and salt transport through the plants: structure and functioning of the xylem. *Plant, Cell Environment* **26**, 87-101.

DesRochers A, Thomas BR (2003) A comparison of pre-planting treatments on hardwood cuttings of four hybrid poplar clones. *New For.* **26**, 17-32.

DesRochers A, van den Driessche R, Thomas BR (2007) The interaction between nitrogen source, soil pH, and drought in the growth and physiology of three poplar clones. *Can J Bot* .**85**, 1046-1057.

Ennajeh M, Tounekti T, Vadel AM, Khemira H, Cochard H (2008) Water relations and drought-induced in olive (*Olea europea*) varieties 'Meski' and "Chemlali" during severe drought. *Tree Physiol.* **28**, 971-976.

Evett SR, Tolk JA, Howell TA (2005) Time domain reflectometry laboratory calibration in travel time, bulk electrical conductivity, and effective frequency. *Valdose Zone J.* **4**, 1020-1029.

Ferris R, Long L, Bunn SM, Robinson KM, Bradshaw HD, Rae A, Taylor G (2002) Leaf stomatal and epidermal cell development: identification of putative quantitative trait loci in relation to elevated carbon dioxide concentration in poplar. *Tree Physiol.* **22**, 633–640.

Galmés J, Pou A, Alsina MM, Tomàs M, Medrano H, Flexas J (2007) Aquaporin expression in response to different water stress intensities and recovery in Ritchter-110 (*Vitis* sp.): relationship with ecophysiological status. *Planta* **226**, 671-681.

Hacke U, Sauter JJ (1995) Vulnerability of xylem to embolism in relation to leaf water potential and stomatal conductance in *Fagus sylvatica* f. *purpurea* and *Populus balsamifera*. *J Exp Bot* **46**, 1177-1183.

Hacke U, Stiller V, Sperry JS, Pittermann J, McCulloh KA (2001) Cavitation fatigue. Embolism and refilling cycles can weaken the cavitation resistance of xylem. *Plant Physiol.* **125**, 779-786.

Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. *Perspect Plant Ecol.* **4**, 97-115.

Hacke UG, Sperry JS (2003) Limits to xylem refilling under negative pressure in *Laurus nobilis* and *Acer negundo*. *Plant Cell Environ.* **26**, 303–311.

Hacke UG, Sperry JS, Wheeler JK, Castro L (2006) Scaling of angiosperm xylem structure with safety to efficiency. *Tree Physiol.* **26**, 689-701.

Hillel D (1998). Content and potential of water. *In:* Hillel D (eds) Environmental soil physics. San Diego CA. Academic press. Pp. 129-172.

Holbrook NM, Zwieniecki MA (1999) Embolism repair and xylem tension: Do we need a miracle*? Plant Physiol.* **120**, 7-10.

Hukin, D., H. Cochard, E. Dreyer, D. Le Thiec and M.B. Bogeat-Triboulot. 2005. Cavitation vulnerability in roots and shoots: does *Populus euphratica* Oliv., a poplar from arid areas of Central Asia, differ from other poplar species? *J Exp Bot.* **56**: 2003-2010.

Hungtington TG (2006) Evidence for intensification of the global water cycle: Review and synthesis. *J. Hydrol.* **319**, 83-95.

Jones HG, Sutherland RA (1991) Stomatal control of xylem embolism. *Plant Cell Environ.* **14**, 607-612.

Johnson H, Thunholm B, Lundin LC (1995) Experimental system for one-dimensional freezing of undisturbed soil profiles. *Soil Technology* **7**, 319-325.

Kaldenhoff R, Ribas-Carbo M, Sans JF, Lovisolo C, Heckwolf M, Uehlein N (2008) Aquaporins and plant water balance. *Plant Cell Environ.* **31**, 658-656.

Kim HS, Oren R, Hinckley TH (2008) Actual and potential transpiration and carbon assimilation in an irrigated poplar plantation. *Tree Physiol.* **28**,559–577.

Larson PR, Isebrands JG (1971) The plastochron index as applied to developmental studies of cottonwood. *Can J For Res.***1**, 1-11.

Lemprière TC, Bernier PY, Carroll AL, Flannigan MD, Gilsenan RP, McKenney DW, Hogg EH, Pedlar JH, Blain D. 2008. The importance of forest sector adaptation to climate change. Information Rerport NOR-X-416E. Canadian Forest Service. Natural Resources Canada.

Maherali H, Reid CD, Polley HW, Johson HB, Jackson RB (2002) Stomatal acclimation over a subambient to elevated $CO_2$ gradient in a $C_3/C_4$ grassland. *Plant Cell Environ.* **25**, 557-566.

Maherali H, Pockman WT, Jackson RB (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* **85**(8), 2184-2199.

Maherali H, Moura CF, Caldeira MC, Willson CJ, Jackson RB (2006) Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees. *Plant Cell Env.* **29**, 571-583.

Malone SR, Mayeux HS, Johnson HB, Polley HW (1993) Stomatal density and aperture length in four plant species grown across a subambient $CO_2$ gradienent. *Am. J.Bot.* **80**, 1413-1418.

Nardini A, Salleo S, Lo Gullo MA, Pitt F (2000) Different responses to drought and freeze stress of *Quercus ilex* L. growing along a latitudinal gradient. *Plant Ecol.* **148**, 1390147.

Pallardy SG, Kozlowski TT (1979) Frequency and length of stomata of 21 *Populus* clones. *Can. J. Bot.* **57**, 2519–2523.

Pammenter NW, Willigen CV (1998) A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiol.* **18**, 589-593.

Pearce DW, Millard S, Bray DF, Rood SB (2006) Stomatal characteristics of riparian poplar species in a semi-arid environment. *Tree Physiol.* **26**, 211-218.

Pinheiro JC, Bates DM (2000) Mixed-Effects Models in S and S-PLUS. New York. Springer. Pp. 304-414.

Pinheiro J, Bates D, DebRoy S, Sarkar D (2007) The R Core Team. nlme: Linear and Nonlinear mixed effects models. R package version 3, Pp. 1-86.

Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *Am J Bot* **87**, 1287-1299.

Ratrowsky DA (1990) Handbook of nonlinear regression models. M. Dekker, New York. USA.

Reich PB. (1984) Leaf stomatal density and diffusive conductance in three amphistomatous hybrid poplar cultivars. *New Phytol* **98**, 231-239.

Reynolds WD, Topp GC (2008) Soil water desorption and imbibition: tension and pressure techniques. In: Carter, M.R. and E.G. Gregorich (eds) *Soil sampling and methods of analysis*. Taylor and Francis, Boca Raton. 1224 pp.

Ritchie GA, Hinckley TM (1975) The pressure chamber as an instrument for ecological research. *Adv Ecol Res*. **9**, 165-254

Rood SB, Zanewich K, Stefura C, Mahoney JM (2000) Influence of water table decline on growth allocation and endogenous gibberellins in black cottonwood. *Tree Physiol.* **2**, 831–83.

Sakr S, Alves G, Morillon R, Maurel K, Decoourteix M, Guilliot A, Fleurat-Lessard P, Julien JL, Chrispeels MJ (2003) Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiol.* **133**, 630-641.

Salleo S, Lo Gullo MA, Raimondo F, Nardini A (2001) Vulnerability to cavitation of leaf minor veins: any impact on leaf gas exchange? *Plant Cell Environ.* **24**, 851–859.

Salleo S, Lo Gullo MA, Trifilò P, Nardini A (2004) New evidence for a role of vessel-associated cells and phloem in the rapid xylem refilling of cavitated stems of *Laurus nobilis* L. *Plant Cell Environ.* **27**, 1065–1076.

SAS (2002) Version 9.1. SAS Institute, Cary NC US.

Schindler DW, Donahue WF (2006) An impeding water crisis in Canada's western prairie provinces. *PNAS.* **103**, 7210-7216.

Scholander PB. Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. *Sci.* **51**, 119-125.

Silim S, Nash R, Reynard D, White B, Shroeder W (2009) Leaf gas exchange and water potential responses to drought in nine poplar (*Populus* spp.) clones with contrasting drought tolerance. *Trees.* **23**, 959-969.

Souch CA, Stephens W (1998) Growth, productivity and water use in three hybrid poplar clones. *Tree Physiol.* **18**, 829-835.

Sparks JP, Black RA (1999) Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. *Tree Physiol.* **19**, 453-459.

Sperry JS (1995) Limitations on stem water transport and their consequences. In: Plant stems: Physiology and functional morphology (ed. BL., Gartner). Academic Press, New York.

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ.* **11**, 35-40.

Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *J Exp Bot.* **44**(263), 1075-1082.

Sperry JS, Hacke UG (2002) Desert shrub water relations with respect to soil characteristics and plant functional type. *Funct Ecol.* **16**, 367-378,

Suzuki S (2004) Verification of freezing point depression method for measuring matric potential of soil water. *Soil Sci Plant Nutr.* **50**, 1277-1280.

Topp GC, Davis JL, Annan AP (1980) Electromagnetic determination of soil water content: Measurements in coaxial transmission lines. *Water Resour Res.* **16**(3), 574-582.

Trenberth KE, Jones PD, Ambenje P, Bojariu R, Easterling D, Tank AK, Parker D, Rahimzadeh F, Renwick JA, Rusticucci M, Soden B, Zhai P. (2007) Observations: Surface and Atmospheric Climate Change. In: *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Tschaplinski TJ, Blake TJ (1989) Water stress tolerance and late-season organic solute accumulation in hybrid poplar. *Can J Bot* .**67**, 1681-1688.

Tschaplinski TJ, Tuskan GA (1994) Water-stress tolerance of black and eastern cottonwood clones and four hybrid progeny. II. Metabolites and inorganic ions that constitute osmotic adjustment. *Can J For Res.* **24**, 681-687.

Tschaplinski TJ, Tuskan GA, Gunderson CA (1994) Water-stress tolerance of black and eastern cottonwood clones and four hybrid progeny. I. Growth, water relations, and gas exchange. *Can J For Res.* **24**, 364-371.

Tyree MT, Dixon MA (1986) Water stress induced cavitation and embolism in some woody plants. *Physiol Plant.* **66**, 397-405.

Tyree MT, Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. *Ann. Rev. Plant Phys. Mol. Bio.* **40**, 19-38.

Tyree MT, Ewers FW (1991) The hydraulic architecture of tress and other woody plants. *New Phytol.* **119**, 345-360.

Tyree MT, Alexander J, Machado JL (1992) Loss of hydraulic conductivity due to water stress in intact juveniles of *Quercus rubra* and *Populus deltoides*. *Tree Physiol.* **10**, 411-415.

Tyree MT, Kolb KJ, Rood SB, Patiňo S (1994) Vulnerability to drought-induced cavitation of riparian cottonwoods in Alberta: a possible factor in the decline of the ecosystem? *Tree Physiol.* **14**, 455-466.

Tyree MT, Salleo S, Nardini A, Lo Gullo MA, Mosca R (1999) Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? *Plant Physiol.* **12**, 11-21.

Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap. *In*: TE Timell (Ed.) Springer Series in wood Science Springer, Germany.

Tyree MT, Engelbrecht BMJ, Vargas G, Kursar TA (2003) Desiccation tolerance of five tropical seedlings in Panama: Relationship to a field assessment of drought-performance. *Plant Physiol.* **132**, 1439–1447

Westhoff M, Schneider H, Zimmermann D, Mimietz S, Stinzing A, Wegner LH, Kaiser W, Krohne G, Shirley St, Jakob P, Bamberg E, Bentrup F-W, Zimmermann U (2008) The

mechanisms of refilling of xylem conduits and bleeding of tall birch during spring. *Plant Biol.* **10**, 604-623.

Wheeler JK, Sperry JS, Hacke UG, Hoang N (2005) Intervessel pitting and cavitation in woody *Rosaceae* and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant Cell Environ.* **28**, 800-812.

Xu Z, Zhou G (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J Exp Bot.* **59**, 3317-3325.

Yamada S. Komori T, Myers PN, Kuwata S, Kubo T, Imaseki H (1997). Expression of plasma membrane water channel genes under water stress in *Nicotiana excelsior. Plant Cell Physiol.* **38**, 1226-1231.

# Chapter Four
# Osmotic adjustments in poplar clones under drought stress

## *4.1 Introduction*

Growth of poplar trees is strongly affected by soil water availability (Giovannelli *et al.*, 2007) and depends on the ability of cells to maintain turgor pressure for cell expansion (Frost *et al.*, 1991). The ability to maintain water balance through osmotic adjustment, cell wall elasticity and increased apoplastic water fraction, plays an important role in the maintenance of leaf turgor and, hence, the ability to withstand water stress (Turner & Jones, 1980; Blake *et al.*, 1991). Under drought stress, both elastic and osmotic adjustments have been reported as the key components in the maintenance of turgor in poplars (Yin *et al.*, 2005; Giovannelli *et al.*, 2007).

Osmotic adjustment occurs by lowering osmotic potential due to accumulation of compatible solutes generated by the uptake, internal production or transfer of osmotically active substances such as sugars and amino acids (Abrams, 1988; Mullet & Whitsitt, 1996). This is one of the common drought resistance mechanisms in many plant species (Morgan, 1984; Gunasekera & Berkowitz, 1992). An accumulation of osmotically-active solutes lowers osmotic potential and may result in a decrease in water potential, thus promoting water uptake from drying soils, and in the maintenance of turgor in plant tissues. The importance of osmotic adjustment in drought stress resistance varies between tree species (Morgan, 1984; Gunasekera & Berkowitz, 1992). It has been demonstrated that in *Ligustrum japonicum*, *Eurya japonica* (Harayama *et al.*, 2006) and *Olea europea* L (Dichio *et al.*, 2005), osmotic adjustment is an important mechanism helping maintain metabolic functions of the plant at low leaf water potential. Similarly, studies in *Populus* have shown evidence of osmotic adjustment in the leaves of various poplar clones (e.g. Tschaplinski & Blake, 1989; Tschaplinski & Tuskan, 1994; Tschaplinski *et al.*, 1998). These studies demonstrated that several poplar hybrids displayed some degree of drought

tolerance by high accumulation of soluble carbohydrates and osmotic adjustment.

Osmotic adjustment enables continuation of cell elongation, maintenance of stomatal opening and photosynthesis under drought (Turner & Jones, 1980). Moreover, as drought stress progresses, sugar concentration may increase due to mobilization of starch stored in the chloroplasts (Nilsen & Orcutt, 1996), although the concentration of carbohydrates depends on the stage of the plant and the degree of drought stress. In addition to osmotic adjustment, proline is involved in prevention of cell membrane damage and protein denaturation (Ain-Lhout *et al.*, 2001). Previous studies have shown a correlation between high levels of proline and plant adaptation to drought and salt stress in poplars (Watanabe *et al.*, 2000; Lei *et al.*, 2006). However, the exact role of proline in osmotic adjustment is not yet fully understood (Marcum, 2002; Ashraf & Foolad, 2007).

In plants that do not adjust osmotically, turgor can also be maintained by increasing tissue elasticity (Kozlowski & Pallardy, 2002), as well as apoplastic water fraction (Turner & Jones, 1980; Blake *et al.*, 1991). However, little is known about turgor maintenance strategies of poplars. The present study was carried out to determine the significance of cell elasticity and osmotic adjustment in drought resistance of several poplar clones. In this study, carbohydrates and amino acid concentrations in leaves and roots were examined in rooted stem cuttings of six hybrid poplar clones and in balsam poplar (*Populus balsamifera*) exposed to drought stress and the pressure-volume (P-V) technique was used to examine the leaf water relations characteristics.

### *4.2 Materials and Methods*

Ten-cm-long stem cuttings of P38P38 (*P. balsamifera* x *P. simonii* cv. P38P38), Walker (*P. deltoides* x (*P.* x *petrowskyana*) cv. Walker), Okanese (Walker x (*P. x petrowskyana*) cv. Okanese), Northwest [*P. balsamifera* x *P. deltoides* cv. Northwest (Jackii hybrid)], Assiniboine (open-pollinated Walker cv. Assiniboine), Berlin (*P. x berolinensis* cv. Berlin) and balsam poplar (*P. balsamifera* L.) were collected in February 2007 from the Alberta-Pacific Forest

Industries Inc. (Al-Pac) experimental field site (54°49'N, 113°31'W), about 200 km northeast of Edmonton, Alberta, Canada. The cuttings were transported to the University of Alberta where they were soaked in water at room temperature for two days prior to planting (DesRochers and Thomas, 2003). Each cutting was rooted in Metro Mix® 290 (Sun Gro Horticulture Vancouver, BC, Canada $^{TM}$) growth medium in Styroblock containers (440 ml volume/cell, Beaver Plastic Ltd. Alberta, Canada) for 45 days and fertilized weekly with a 200 mg $L^{-1}$ solution of 15-30-15 (N-P-K) fertilizer (Plant Products Company Ltd., Brampton Ont.). Rooted cuttings were transferred to 4.5 L (6" x 7") plastic pots filled with homogenized Metro Mix® 290 amended with 2.73 $gL^{-1}$ Nutricote® 13-13-13 100 d slow-release fertilizer (6.5% $NO_3$-N, 6.5 $NH_4$-N, 13% $P_2O_5$, 13% $K_2O$, 1.2% Mg, 0.02% B, 0.05% Cu, 0.2% chelated Fe, 0.06% Mn, 0.02% Mo, 1.3% EDTA; Plant Products Company Ltd.). The plants were grown and watered daily in a greenhouse under the following conditions: 22°C/18°C (day/night) temperature, relative humidity of 20-35% and under natural light supplemented with incandescent lamps to maintain 16-h photoperiod.

### *4.2.1 Treatments*

Drought treatments were applied to plants approximately three months after rooting. All pots were saturated with water and allowed to dry until a desired level of drought was achieved. The following treatments were applied: mild drought (≈ 25% soil water content, SWC), severe drought (≈ 12% SWC), severe drought followed by 30 minutes of recovery (rewatering to >40% SWC), severe drought followed by two days of recovery (rewatering to >40% SWC), and well-watered control plants (>40% SWC). Severe drought produced leaf wilting and some leaf necrosis but did not affect plant survival. Soil water content (SWC) was measured daily using time-domain reflectometry (TDR) (Topp *et al.*, 1980).

### *4.2.2 Experimental design and measurements*

#### *4.2.2.1 Non-structural carbohydrates (starch and soluble sugars)*

This experiment used a completely randomized block design with two treatment factors (drought and clone) with five drought treatment levels (described in section 3.2.1) and seven poplar clones. Each drought treatment was applied to three trees per clone in two blocks. Blocking was in time being carried out each week for two weeks. On each day, a single treatment *versus* its control was measured on the seven poplar clones.

Non-structural carbohydrate concentrations were examined in three-month-old rooted poplar cuttings exposed to different drought and recovery treatments. Leaf plastochron index (LPI) (Larson & Isebrands, 1971) was used to identify the leaves (LPI 11) that were taken for the analysis. Also, samples with fine new roots were collected between 11.00 and 14.00 h, immediately frozen in liquid nitrogen and kept at -80°C until the analysis. Two months later, the samples were oven-dried at 68°C (Chow & Landhäusser, 2004) for 2-3 days, ground in a Wiley mill (40-mesh) and stored in airtight containers at room temperature.

Sugars were extracted from tissue samples containing approximately 50 mg of dry weight with 80% hot ethanol. Sugar concentrations were then measured spectrophotometrically using the phenol-sulfuric acid reagent (Chow & Landhäusser, 2004) (Beckman Model DU-600 spectrophotometer, Beckman instrument Inc., CA) at 490 nm. Starch, remaining in the residue was subsequently solubilised with 0.1 N NaOH, hydrolyzed with an enzyme mixture of $\alpha$-amylase and amyloglucosidase, and then measured colorimetrically at 525 nm using a peroxidise-glucose oxidase-o-dianisidine solution (Chow & Landhäusser, 2004).

#### *4.2.2.2 Proline and total amino acids*

This experiment used a completely randomized block design with two treatment factors (drought and clone) with five drought treatment levels (described in section 3.2.1) and seven poplar clones. Each drought treatment

was applied to three trees per clone in two blocks. Blocking was in time being carried out each week for two weeks. On each day, a single clone with all treatments was measured.

For proline determination, approximately 0.15 g DW tissue was homogenized in 5 ml of 3% (w/v) sulfosalicylic acid and filtered through a 1 µm filter. To 600 µl of the filtrate, 600 µl of acid ninhydrin was added (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) and boiled for 1 h at 100°C (Bates *et al.*, 1973; Meloni *et al.*, 2004). The reaction was stopped by cooling the tubes in a water bath at room temperature (25°C) for 10 min. The absorbance was measured at 520 nm using the Beckman Model DU-600 spectrophotometer (Beckman instrument Inc., CA), and the concentration of proline was estimated by referring to a standard curve prepared using L-proline. Tissue concentrations of proline were expressed as µg $g^{-1}$ dry weight (DW).

The quantitative measurement of the total free amino acids of the supernatant was performed using the ninhydrin reaction described by Correia *et al.*, (2005) and Lei *et al.*, (2006). To 600 µl of buffered ninhydrin solution, 600 µl of the supernatant was added and heated in a boiling water bath for 15 min. The mixture was then cooled in a water bath at room temperature and 900 µl of 50% ethanol was added. Absorbance at 570 nm was determined with a spectrophotometer using *L*-arginine as a standard.

#### *4.2.2.3 Pressure-volume assay*

The experiment was a completely randomized design with well-watered plants from seven poplar clones. Plants were regularly moved to avoid an edge effect with a minimum of six plants from each poplar clone taken for the pressure-volume analysis.

One day prior to sampling, trees were watered to saturation. One upper branch from each plant were excised under water, placed in distilled water and kept in the dark overnight to ensure full hydration. The following day, leaf nine (LPI 9) (Larson & Isebrands, 1971) was excised under water from each branch and used to generate a pressure-volume curve with the Scholander-type pressure chamber (Scholander *et al.*, 1965; Tyree & Hammel, 1972). Detached

leaves were initially weighed to determine turgid weight, and then gradually dehydrated using a free transpiration method (Hinckley *et al.*, 1980). Leaf water potentials at different points of hydration were measured with a pressure chamber (PMS Instruments Co., Corvallis, OR, USA). High humidity in the pressure chamber was maintained with a moist paper towel to prevent water loss (Turner, 1981). Leaf fresh weights were used to calculate changes in leaf water content. For water potential measurements, the pressurization rate was approximately 0.025 MPa $s^{-1}$ (Turner, 1981). After the last set of water potential measurements, the leaves were oven-dried at 80°C for 48 h to determine their dry weights.

### *4.3.3 Statistical analysis*

#### *4.3.3.1 Non-structural carbohydrates, proline and total amino acids*

Each sample was quantified in duplicate for two spectrophotometric readings and three to six samples of each clone per tissue per treatment combination were analyzed. Analysis of variance (ANOVA) procedure was performed using the *Proc Mixed* procedure of SAS (9.1) (SAS Institute, Cary NC US, 2002). The assumption of normality was assessed using the Proc UNIVARIATE function with the Kolmogorov-Smirnov test for normality. Treatment and clone effects were analyzed using one way analysis of variance performed by Proc mixed procedure. Data were log-transformed for root amino acids under recovery after two days, and leaf amino acids for balsam poplar, to satisfy assumptions of normality and homogeneity. Least squared means were separated using *pdiff* option, and $\alpha$=0.05 was used to determine whether there were any differences between clones and treatments. Linear models and ANOVA tables for each analysis are shown in Appendix D.

#### *4.3.3.2 Pressure-volume curves (P-V)*

The P-V curve was obtained by plotting $1/\Psi_x$ versus $R^*$ (type II transformation, see Tyree & Ritchter 1981, 1982; Andersen *et al.*, 1991) (Fig. 4-1), and the estimates of pressure-volume parameters were obtained as described below. The various components of water relations were calculated by developing

a P-V curve based on six to eight replicates for each clone. Estimates of osmotic potential at full turgor and at the turgor loss point, modulus of elasticity at full turgor (ε), and relative water content at zero turgor ($R^*_0$), and symplastic water fraction ($R^*_s$) were obtained as described by Proctor *et al.*, (1998). The extrapolation of a straight line provided an estimate of the osmotic potential at full hydration ($\pi_{ft}$) (Fig. 4-1. pt B), and the osmotic potential at zero turgor ($\pi_{TLP}$) (Fig. 4-1. pt C), was estimated from the point at which water potential and osmotic potential were equal (Tyree *et al.*, 1972; Turner, 1981). The *y*-intercept of the linear regression estimated the reciprocal of $\pi_{ft}$, and the *x*-intercept was taken as an estimate of the effective osmotic volume of the symplast (Proctor *et al.*, 1998).

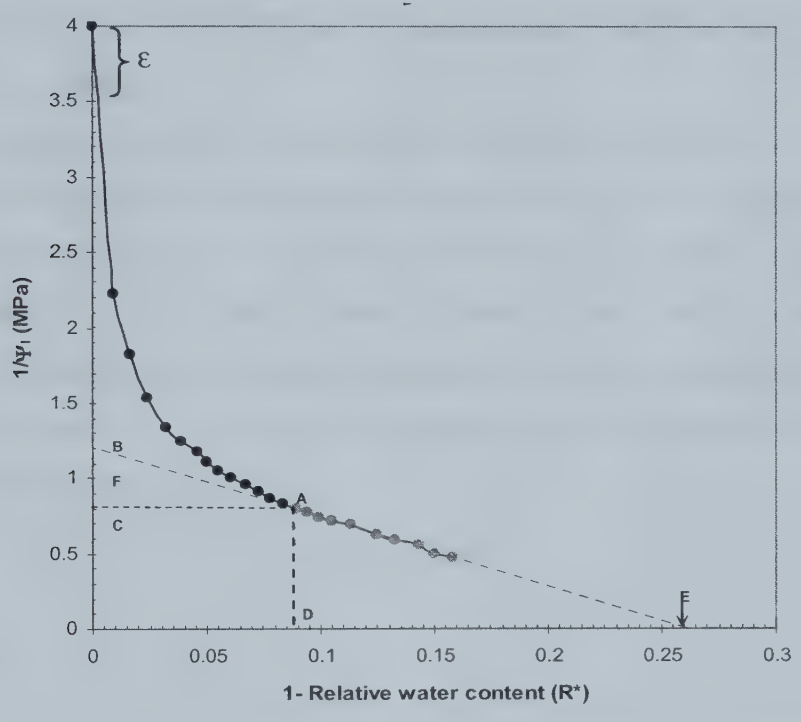



**Figure 4-1.** Representative plot of inverse balancing pressure of a poplar leaf versus relative water content (R*). The data are from one balsam poplar leaf. The full line was obtained from the non linear model. The linear regression, from the non-turgid region, is as follows: $y$=-2.141 $x$ + 0.9467 (with r=0.96). Where A: turgor loss point, B: osmotic potential at full hydration ($\pi_{ft}$), C: osmotic potential at the turgor loss point ($\pi_{TLP}$), D: relative water content at the turgor loss point, E: relative water content of the symplast, and F: osmotic amplitude for turgor maintenance. ε= elastic modulus.

Relative water content was calculated from the equation:

$$R^* = [(FW - DW) / (TW - DW)] * 100 \quad [7]$$

where TW is the weight of the saturated leaf, FW is the fresh weight immediately after the water potential determination, and DW is the constant dry weight obtained after the end of measurements.

Maximum tissue bulk modulus of elasticity ($\varepsilon_{max}$) was determined from the maximum slope of a linear regression line between the turgor potential ($\Psi p$) and relative symplastic water content at full saturation; where $\Psi p$ decreased linearly with the decrease in relative symplastic water content (Stadelmann, 1984).

$$\varepsilon = \frac{\Delta P}{\Delta R^*/R^*} \qquad [8]$$

where P is the bulk turgor potential and $\Delta R^*/R^*$ represents the differential change in tissue relative water content.

Analysis of variance (ANOVA) procedure was performed using the *Proc Mixed* procedure of SAS (9.1) (SAS Institute, Cary NC US). The assumption of normality was assessed using the Proc UNIVARIATE function with the Kolmogorov-Smirnov test for normality. Least squared means were separated using pdiff option, and $\alpha$=0.05 was used to determine whether there were any differences between clones in the P-V parameters. ANOVA tables for each analysis are shown in Appendix D.

## *4.3 Results*

### *4.3.1 Non-structural carbohydrates in leaves and roots*

Sugar concentrations in leaves were similar in all treatments (Table 4-1). With the exception of Northwest clone were a significant increase (p=0.004) in sugar concentration was observed under mild drought compared with well watered plants. In roots, sugar concentrations were also similar in all examined poplars clones that were exposed to the different drought treatments. There was a significant decrease (ca. 37%) (p=0.006) in sugar content in severely-droughted Okanese compared with well-watered control (Table 4-2). Also,

significant increases (ca. 27%) (p=0.02) were observed in Northwest clone after two days of rewatering (Table 4-2).

In general, well-watered plants of different clones had different leaf starch concentrations. Okanese had the highest leaf starch concentration and Northwest and balsam had the lowest concentrations in all of the examined clones (Fig. 4-2). Leaf starch content in P38P38 exposed to mild (p=0.002) and severe drought (p=0.01) increased markedly compared with well-watered plants (Fig. 4-2B). For the remaining poplar clones, leaf starch content was not significantly different between treatments (Fig. 4-2).

### *4.3.2 Total amino acids and proline concentrations*

Total amino acid concentrations varied among clones and tissues. Drought treatments had little effect on the concentrations of leaf amino acids, with the exception of Walker where increases of almost three-fold were observed under severe drought compared with well-watered control (p=0.03) (Table 4-3). In roots, amino acid concentration significantly increased in Assiniboine exposed to mild and recovery treatment compared with control plants (p=0.006) (Fig. 4-3A). Amino acid concentrations in P38P38 were similar in all the treatments. On the contrary, amino acid significantly increased in Northwest roots exposed to severe drought and two days after rewatering (p=0.01) (Fig. 4-3C). Changes in amino acid concentration were not observed in Okanese (Fig. 4-3D). Rewatering treatment in Balsam induced an increase of amino acids in roots of more than two folds compared with control plants (p=0.05) (Fig. 4-3E). Walker clones exposed to severe and recoveries after 30 minutes and two days signifincatly increased root amino acid concentration (p=0.002) (Fig. 4-3F).

Proline concentration in leaves was not affected by the different drought treatments in most of the clones, with the exception of Walker (p=0.003) which showed an almost two-fold increase in response to mild stress and two-day recovery compared with well-watered control (Table 4-4). In roots, there were no significant differences in proline concentrations under the drought treatments with the exception of Walker (p=0.0005) (Fig. 4-4F). Recovery treatment after two days induced an increase in proline concentration in roots for Northwest

(p=0.005), Walker (p=0.0005) and almost a two-fold increase in balsam poplar (p=0.02) compared with controls (Fig. 4-4).

### *4.3.3 Leaf water parameters*

Pressure-volume analyses of poplar leaves showed that most of the poplar clones have similar values of osmotic potential at full hydration ($\pi_{ft}$). Walker and Berlin had significantly higher $\pi_{ft}$ values compared with Northwest (p=0.006). The values of turgor loss point ($\pi_{TLP}$) were above -1.5 MPa, with the highest values (least negative) in all of the examined clones present in Walker (-0.92 ± 0.04 MPa). In contrast, Assiniboine had the lowest value of $\pi_{TLP}$ (-1.4 ± 0.06 MPa), followed by P38P38 (-1.3 ± 0.05 MPa) (Table 4-5). Water saturation deficit at turgor loss point $(1\text{-RWC})_{TLP}$ was significantly different between the clones (p=0.005), with the highest value found in Northwest (Table 4-5).

The symplastic fraction in poplars leaves varied between 25 to 50% of the total tissue water content. This variable did not differ to a great extent between the clones except for Northwest which had an almost two-fold higher symplastic fraction value compared with the remaining clones (Table 4-5). Bulk modulus of elasticity was slightly different between the examined clones (Table 4-5) with the highest values of $\varepsilon_{max}$ (less elastic) present in Northwest (14.7±0.51) and the lowest values found in Berlin (11.3±0.65), Okanese (11.57 ± 0.88) and P38P38 (11.64 ± 0.55), respectively.

## *4.4 Discussion*

Although drought treatments did not have a significant effect on leaf carbohydrate concentrations in most of the studied poplar clones, P38P38 had a higher leaf starch content when exposed to mild and severe drought stress compared with well-watered control. Increased leaf starch levels in P38P38 may be helpful as energy reserves during drought recovery. However, in contrast to other studies (Tschaplinski & Blake, 1989; Tschaplinski & Tuskan, 1994; Gebre *et al.*, 1994), it appears that soluble carbohydrates did not appear to play a major role in osmotic adjustment of the studied poplar clones. High variability in root

starch content in this study could be due to differences in carbohydrate translocation rates in different poplar clones. Likewise, Pregitzer & Friend (1996) reported that allocation of carbon to *Populus* roots seems to be controlled by changes in the environment.

Amino acids play important roles during drought stress due to their roles in osmotic adjustment (Ranieri *et al.*, 1980; Yin *et al.*, 2009), as the primary products of inorganic nitrogen assimilation, and the precursors of proteins and nucleic acids. In the present study, changes in total amino acid concentrations by drought varied between the clones and examined tissues. There were no changes in the amino acid concentrations in leaves with the exception of the Walker clone where the amino acid content increased under severe drought, likely playing a role in osmoregulation or as anitrogen reserve.. In contrast, no changes in amino acids for the rest of the studied clones are in agreement with the earlier studies in poplars (Bélanger *et al.*, 1990; Griffin *et al.*, 1991) where amino acid content is generally lower and organ-dependant. In comparison, increases of amino acids concentration in roots, after two days of re-watering, might be a response of recovery capacity of these poplars, hence inducing an increase in the plant metabolism.

The accumulation of proline in response to a variety of abiotic stresses has been often reported for plants (Hare & Cress, 1997). Although proline accumulation in Walker leaves was too low to be solely responsible for osmotic adjustment, it could either be part of turgor maintenance at low water potentials, or play a role in protecting cell functions under stress. During drought recovery, proline breakdown generates reducing equivalents that support mitochondrial oxidative phosphorylation (Hare & Cress, 1997). Therefore, increases in proline concentrations in Walker leaves could be helpful with the maintenance of stomatal opening and photosynthesis of plants after re-watering (Tables 3-1, 4-5). Re-watering treatment caused an increased biosynthesis of total amino acids and proline in Assiniboine, Okanese, Balsam and Walker. Accumulation of proline upon relief from stress in this study is corroborated by the observations of cause-and-effect relationship between proline levels and osmotic tolerance in plants under abiotic stress (Hare & Cress, 1997). Likewise, Sivaramakrishnan (1988) suggested that the role of proline might be in the intermediate recovery of plants after the stress. Accumulation of proline was dependant on the clone and

tissue. In line with these results, Watanabe *et al.* (2000) showed that differences in proline accumulation depended on poplar clone and tissue type when plants were subjected to salt and osmotic stresses.

The results obtained with the P-V technique indicate that for most of the poplars, high cell wall elasticity may help the plants maintain cell turgor pressure at lower tissue water contents. Greater elasticity prevents the rupture of the cell wall and injury of the plasma membrane (Zimmermann & Steudle, 1978). Conversely, Northwest showed low cell wall elasticity and developed low leaf water potential (see Table 3-1, Chapter 3), thus, having a better ability for maintaining a water potential gradient for water uptake from drying soil without undergoing large tissue water deficits (Abrams, 1988). Plants with low cell wall elasticity also have greater resistance to deformation of the cell walls and the ability of the cells to retain water (Grossnickle, 2000). The particularly low values of symplastic fraction obtained in the studied poplars have been previously reported (Tschaplinski *et al.*, 1994). One possible explanation of these low values is that drought acclimation stimulated increases in the apoplastic water fraction in order to induce turgor maintenance (Radin, 1983; Sattelmacher, 2000; Saito *et al.*, 2006). Apoplastic water might act as buffer for cell water during drought stress, and there appears to be a large variation in apoplastic water fraction among species (Tyree & Jarvis, 1982). As suggested by Nilsen & Orcutt (1996), when apoplastic water fraction is greater than the symplastic fraction, solutes are held in a smaller proportion of tissue water; thus, turgor potential is maintained using small amount of solutes. In addition, the lower values of osmotic potential at full hydration for Northwest clone might have promoted water uptake in this clone under drought stress.

In conclusion, as drought developed, different strategies of drought resistance were used by the different examined poplar clones. Walker plants showed osmotic adjustment by increasing solute concentrations in leaves and roots (Table 4-6). Osmotic adjustment could help maintain water uptake and protect cell structures from damage (Schobert & Tschesche, 1978). However, sugars and amino acids did not appear to play a major role in this response. Osmotic adjustment could be due to accumulation of other solutes (*i.e.* inorganic solutes, Patakas *et al.*, 2002; Kusaka *et al.*, 2005). Alternatively, it could be also attributable to the relatively rapid rate of soil drying in the greenhouse experiment

(Abrams, 1988; Turner & Jones, 1980). On the other hand, increases in starch accumulation in the leaves of P38P38 poplar clone might be due to increased water use efficiency and lowering metabolism to reduce demand for carbon. Starch accumulation in this poplar clone could also facilitate the use of available reserves in plant recovery after re-watering treatment. As shown previously in Chapter 3, Northwest and balsam poplars were relatively resistant to xylem cavitation, thus accumulation of compatible solutes may not be a major drought resistance strategy in these clones. The observed significant accumulation of total amino acid and proline in roots after two days of rewatering for balsam, Assiniboine, Okanese and Walker could probably facilitate drought recovery due to a greater availability of carbon and nitrogen reserves.

**Table 4-1.** Sugar concentrations in poplar leaves of plants subjected to mild and severe drought stress and stress recovery (Means ± SE, n=3-6). C-M: control for mild stress, C-S: control for severe stress, C-R 30 min: control for recovery after 30 minutes of rewatering, C-R 2 days: control for recovery after two days of rewatering, Rec. 30 min: recovery after 30 minutes of rewatering, Rec. 2 days: recovery after 2 days of rewatering. Asterisks denote statistical differences at $p \leq 0.05$ (**) and $p \leq 0.001$ (***)

| | Assiniboine | P38P38 | NW | Okanese | Berlin | Balsam | Walker |
|---|---|---|---|---|---|---|---|
| | | | | Sugar leaves (%DW) | | | |
| C-M | 13.9 ± 1.81 | 19.5 ± 2.09 | 16.7 ± 0.77 ** | 18.5 ± 1.53 | 16.6 ± 1.44 | 13.6 ± 0.78 | 19.0 ± 1.41 |
| Mild | 14.5 ± 1.57 | 15.4 ± 0.59 | 20.0 ± 0.66 | 20.2 ± 1.85 | 15.7 ± 0.59 | 14.3 ± 0.93 | 17.4 ± 1.25 |
| C-S | 17.7 ± 2.03 | 18.8 ± 1.53 | 20.1 ± 0.69 | 19.2 ± 1.03 | 16.6 ± 1.44 | 19.1 ± 0.88 | 15.5 ± 1.28 |
| Severe | 14.7 ± 1.23 | 19.6 ± 1.41 | 18.8 ± 1.15 | 19.3 ± 0.61 | 15.3 ± 1.28 | 16.1 ± 1.51 | 14.5 ± 0.85 |
| C-R 30 min | 16.7 ± 1.29 | 22.6 ± 0.64 | 17.6 ± 1.36 | 20.1 ± 0.99 | | 14.2 ± 1.18 | 18.1 ± 0.93 |
| R. 30 min | 16.7 ± 1.58 | 24.1 ± 1.45 | 19.8 ± 1.03 | 21.7 ± 0.67 | | 16.6 ± 3.32 | 17.3 ± 1.95 |
| C-R 2 days | 13.5 ± 1.64 | 24.3 ± 1.93 | 19.3 ± 1.33 | 19.6 ± 0.81 | 16.6 ± 1.44 | 19.6 ± 3.73 | 17.4 ± 1.52 |
| R. 2 days | 15.3 ± 1.04 | 19.1 ± 2.0 | 19.5 ±1.03 | 18.6 ± 0.62 | 16.7 ± 1.60 | 15.9 ± 0.97 | 18.8 ± 1.12 |

**Table 4-2**. Sugar concentration in poplar roots of plants subjected to mild and severe drought stress and stress recovery (Means ± SE, n=3-6). C-M: control for mild stress, C-S: control for severe stress, C-R 30 min: control for recovery after 30 minutes of rewatering, C-R 2 days: control for recovery after two days of rewatering, Rec. 30 min: recovery after 30 minutes of rewatering, Rec. 2 days: recovery after 2 days of rewatering. Asterisk corresponds to statistically significant differences between treatments for each clone compared with its control. Asterisks denote statistical differences at $p \leq 0.05$ (**) and $p \leq 0.001$ (***)

| | Assiniboine | P38P38 | NW | Okanese | Walker |
|---|---|---|---|---|---|
| | | | Sugar roots (%DW) | | |
| C-M | 5.52 ± 1.01* | 4.81 ± 0.34 | 5.08 ± 0.82 | 6.44 ± 0.43 | 4.92 ± 0.38 |
| Mild | 5.06 ± 0.24 | 4.53 ± 0.32 | 5.86 ± 0.45 | 6.64 ± 0.64 | 5.03 ± 0.59 |
| C-S | 4.97 ± 0.90 | 4.81 ± 0.34 | 4.78 ± 0.49 | 7.59 ± 0.50 ** | 4.31 ± 0.84 |
| Severe | 4.80 ± 0.35 | 5.14 ± 0.35 | 4.84 ± 0.82 | 4.82 ± 0.18 | 5.97 ± 0.49 |
| C-R 30 min | 4.72 ± 0.12 | 4.81 ± 0.34 | 4.83 ± 1.49 | 5.84 ± 0.16 | 4.30 ± 0.86 |
| Rec. 30 min | 4.99 ± 0.23 | 3.91 ± 0.30 | 5.10 ± 0.29 | 5.62 ± 0.25 | 4.11 ± 0.25 |
| C-R 2 days | 4.70 ± 0.60 | 4.81 ± 0.34 | 5.86 ± 0.11 ** | 6.34 ± 0.50 | 6.13 ± 0.00 |
| Rec. 2 days | 5.54 ± 0.42 | 4.52 ± 0.53 | 3.90 ± 0.31 | 8.02 ± 0.36 | 5.18 ± 0.21 |

**Table 4-3.** Amino acid concentration in poplar leaves of plants subjected to mild and severe drought stress and stress recovery for two days (Means ± SE, n=3-6). P-values correspond to statistically significant differences between treatments by clone. Different letters after the values indicate significant differences between treatments

| Clone | Control | Mild | Severe | Rec. 2 days | p-value |
|---|---|---|---|---|---|
| | Amino acids in leaves (mg $g^{-1}$ DW) | | | | |
| Assiniboine | 15.33±1.92 | 16.00±2.93 | 16.78±2.96 | 17.54±2.43 | 0.95 |
| P38P38 | 11.40±0.81 | 10.57±0.69 | 10.72±0.90 | 10.06±0.44 | 0.74 |
| Northwest | 8.04±0.91 | 9.04±1.34 | 9.73±1.58 | 8.72±0.96 | 0.76 |
| Berlin | 7.98±0.27 | 8.60±0.35 | 8.62±0.98 | 6.60±0.29 | 0.12 |
| Balsam | 9.03±1.00 | 9.63±0.51 | 11.92±1.25 | 13.20±3.15 | 0.68 |
| Walker | $6.83±0.26^{b}$ | $19.97±0.7^{a}$ | $7.68±0.07^{ab}$ | $10.43±3.90^{ab}$ | 0.03 |

**Table 4-4.** Proline concentrations in poplar leaves of plants subjected to mild and severe drought stress and stress recovery for two days (Means ± SE, n=3-6). P-values correspond to statistically significant differences between treatments by clone. Different letters after the values indicate significant differences between treatments

| Clone | Control | Mild | Severe | Rec. 2 days | p-value |
|---|---|---|---|---|---|
| | Proline in leaves (µg/g DW) | | | | |
| Assiniboine | 658.89 ± 72.35 | 786.04 ± 75.53 | 726.50 ± 120.21 | 840.21 ± 115.07 | 0.61 |
| P38P38 | 919.62 ± 62.01 | 832.45 ± 63.16 | 883.87 ± 57.05 | 867.51 ± 49.95 | 0.79 |
| Northwest | 401.10 ± 48.72 | 446.57 ± 68.69 | 383.75 ± 40.94 | 363.77 ± 14.30 | 0.62 |
| Berlin | 602.40 ± 40.25 | 578.75 ± 22.02 | 562.36 ± 20.82 | 567.68 ± 25.50 | 0.89 |
| Balsam | 322.35 ± 35.81 | 429.95 ± 24.12 | 465.66 ± 34.62 | 498.56 ± 66.20 | 0.31 |
| Walker | $453.40 ± 5.31^{b}$ | $830.62 ± 60.86^{a}$ | $638.01 ± 2.76^{ab}$ | $724.18 ± 28.53^{a}$ | 0.003 |

**Table 4-5.** Relative water content at turgor loss point [$(RWC)_{TLP}$], symplastic fraction, and maximum bulk modulus of elasticity ($\varepsilon_{max}$), osmotic potential at full hydration ($\pi_{ft}$) and osmotic potential at turgor loss point ($\pi_{TLP}$) of poplar clones (Means ± SE, n=6-8). Different letters after the values indicate significant differences between poplar clones. See text for an explanation of the meaning of the parameters

| Clone | $(RWC)_{TLP}$ (%) | Symplastic fraction | $\varepsilon_{max}$ (MPa) | $\pi_{ft}$ | $\pi_{TLP}$ |
|---|---|---|---|---|---|
| Assiniboine | 10.12 ± 0.51 ab | 0.30 ± 0.028 b | 12.47 ± 0.47 ab | -0.9±0.03 ab | -1.4±0.06 a |
| P38P38 | 7.81 ± 0.73 bc | 0.28 ± 0.024 b | 11.64 ± 0.55 b | -0.89±0.03 ab | -1.3±0.05 a |
| Northwest | 11.07 ± 1.09 a | 0.50 ± 0.048 a | 14.70 ± 0.51 a | -0.97±0.04 a | -1.2±0.05 ab |
| Okanese | 8.37 ± 0.39 abc | 0.26 ± 0.026 b | 11.57 ± 0.88 b | -0.81±0.08 ab | -1.19±0.07 ab |
| Berlin | 8.00 ± 0.67 bc | 0.21 ± 0.018 b | 11.30 ± 0.65 b | -0.75±0.04 b | -1.18±0.09 ab |
| Balsam | 6.78 ± 0.68 c | 0.25 ± 0.023 b | 13.65 ± 0.59 ab | -0.83±0.03 ab | -1.14±0.03 ab |
| Walker | 6.98 ± 0.51 c | 0.30 ± 0.034 b | 12.20 ± 0.92 ab | -0.7±0.03 b | -0.92±0.03 b |

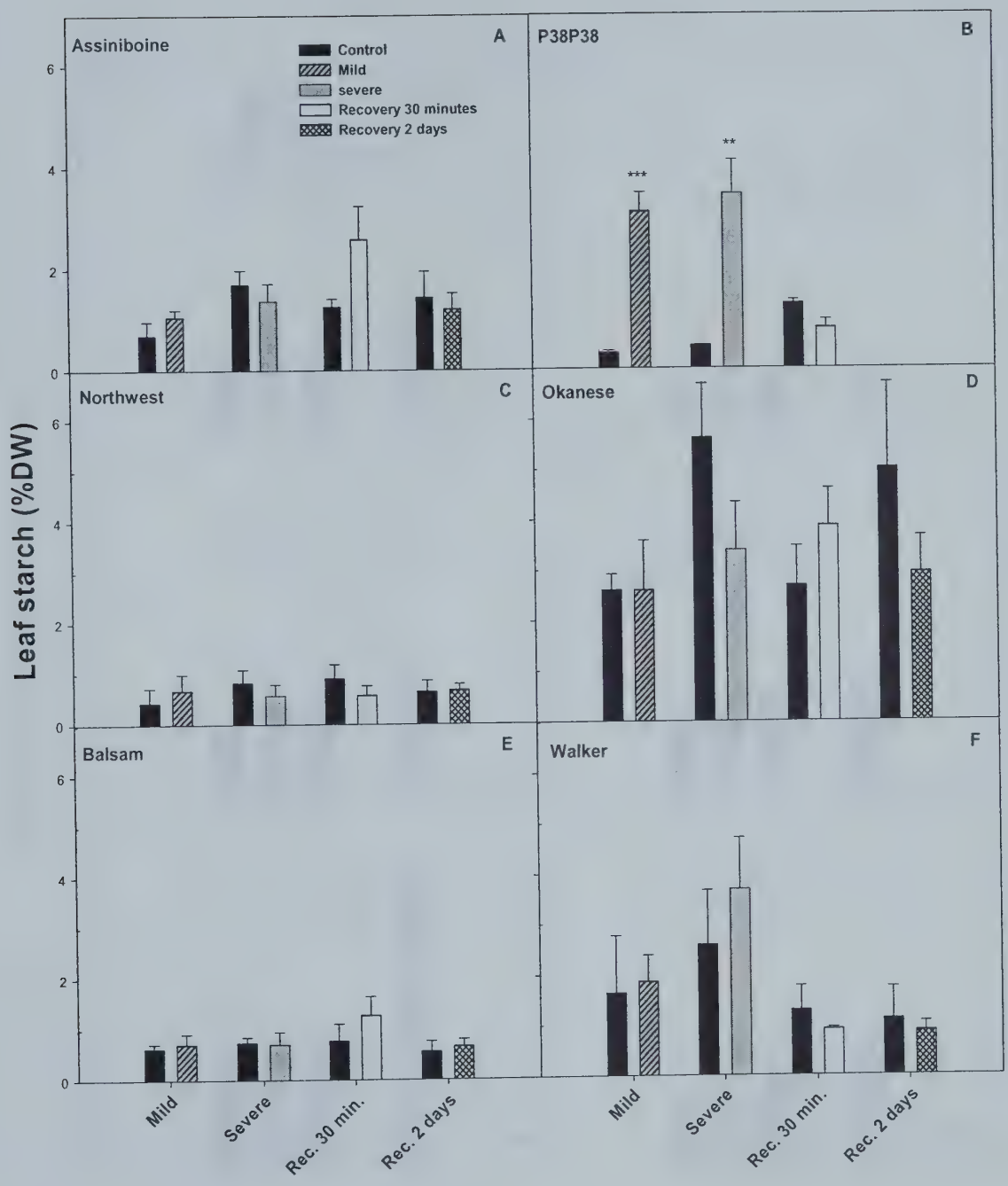



**Figure 4-2.** Starch concentrations in leaves of different poplar clones subjected to mild drought, severe drought, drought recovery for 30 minutes and drought recovery for two days. (A) Assiniboine, (B) P38P38, (C) Northwest, (D), Okanese, (E) Balsam and (F) Walker. Means (n=3-6) + SE are shown. Asterisks denote statistical differences at $p \leq 0.05$ (**) and $p \leq 0.001$ (***).

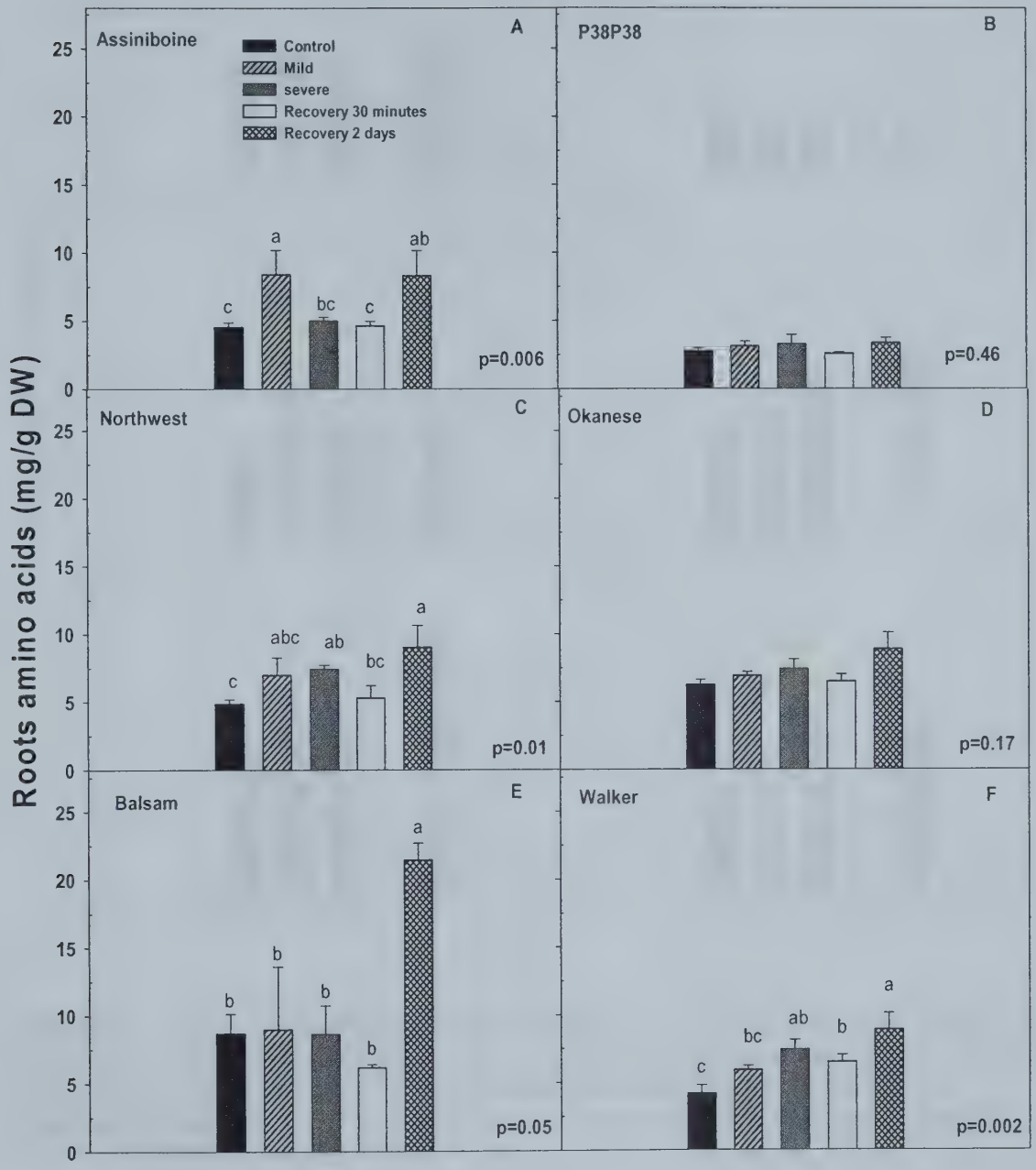



**Figure 4-3.** Amino acid concentrations (means + SE, n=3-6) in roots of different poplar clones subjected to mild drought, severe drought stress, drought recovery for 30 minutes and drought recovery for two days. (A) Assiniboine, (B) P38P38, (C) Northwest, (D), Okanese, (E) Balsam and (F) Walker. Different letters denote statistically difference between treatments for each clone.

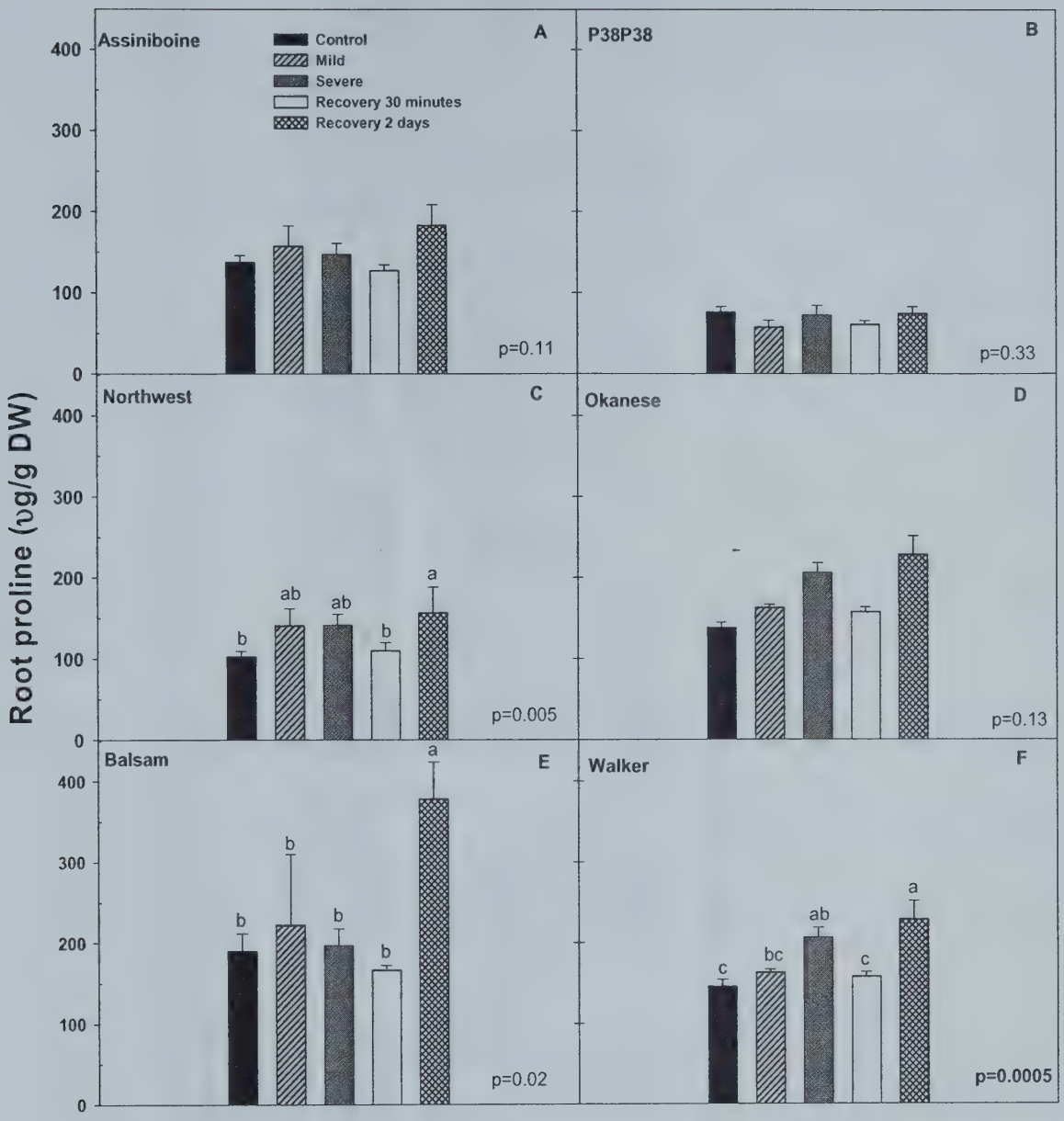



**Figure 4-4.** Proline concentrations (means+ SE, n=3-6) in roots of different poplar clones subjected to mild drought, severe drought stress, drought recovery for 30 minutes and drought recovery for two days. (A) Assiniboine, (B) P38P38, (C) Northwest, (D), Okanese, (E) Balsam and (F) Walker. Different letters denote statistically difference between treatments for each clone.

**Table 4-6.** Summary of the physiological responses of the poplar clones under severe drought stress. Putative drought resistance classification was based on the combination of responses showed in this study and the chapter 3. (↓) Decrease, (↑) increase or (↔) no significant changes compared with control plants

| Poplar clone | Starch leaves* | Amino acid leaves* | Proline leaves* | Amino acid roots* | Proline roots* | Cell wall elasticity | Osmotic potential at full hydration | Osmotic potential at turgor loss point | Putative drought resistance |
|---|---|---|---|---|---|---|---|---|---|
| **Assiniboine** | (↔) | (↔) | (↔) | (↔) | (↔) | | -0.9 | -1.4 | Low |
| **P38P38** | 660% (↑) | (↔) | (↔) | (↔) | (↔) | high | -0.89 | -1.3 | Moderate |
| **Northwest** | (↔) | (↔) | (↔) | 52% (↑) | (↔) | low | -0.97 | -1.23 | Moderate |
| **Okanese** | (↔) | (↔) | (↔) | (↔) | (↔) | high | -0.81 | -1.19 | Moderate |
| **Berlin** | | (↔) | (↔) | | | high | -0.75 | -1.19 | Low |
| **Balsam** | (↔) | (↔) | (↔) | (↔) | (↔) | | -0.84 | -1.14 | Moderate |
| **Walker** | (↔) | 316% (↑) | 40% (↑) | 77% (↑) | 42% (↑) | | -0.70 | -0.92 | Moderate |

* Estimated percentage of decrease or increase compared with control plants

## References


Abrams MD (1988) Sources of variation in osmotic potentials with special reference to North American tree species. *For Sci.* **34**, 1030-1046.

Ain-Lhout F, Zunzunegui M, Diaz Barradas M.C., Tirado R., Clavijo A., Garcia Novo F (2001) Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit. *Plant Soil* **230**, 175-183.

Andersen MN, Jenesen CR, Lösh R (1991) Derivation of pressure-volume curves by non-linear regression procedure and determination of apoplastic water. *J Exp Bot* **42**, 159-165.

Ashraf M, Foolad MR (2007) Roles of glycine, betaine and proline in improving plant abiotic stress resistance. *Env Exp Bot.* **59**, 206-216.

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* **39**, 205-207.

Bélanger RR, Manion PD, Griffin DH (1990) Amino acid content of water-stressed plantlets of *Populus tremuloides* in relation to clonal susceptibility to *Hypoxylon mamatum in vitro*. *Can J Bot* .**68**, 26-29.

Blake TJ, Bevilacqua E, Zwiazek JJ (1991) Effects of repeated stress on turgor pressure and cell elasticity changes in black spruce seedlings. *Can J For Res***21**, 1329-1333.

Dichio B, Xyloyannis C, Sofo A, Montanaro G (2005) Osmotic regulation in leaves and roots of olive trees during a water deficit and rewatering. *Tree Physiol.* **26**, 197-185.

Correia I, Nunes A, Duarte LF, Barros A, Delgadillo I (2005) Sorghum fermentation followed by spectroscopic techniques. *Food Chem.* **90**, 853-859.

Chen S, Wang S, Altman A, Hüttermann A (1997) Genotypic variation in drought tolerance of poplar in relation to abscisic acid. *Tree Physiol.* **17**, 797-803.

Chow PS, Landhäusser SM (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiol.* **24**, 1129-1136.

DesRochers A, van den Driessceh R, Thomas BR (2007) The interaction between nitrogen source, soil pH, and drought in the growth and physiology of three poplar clones. *Can J Bot* .**85**, 1046-1057.

Frost DL, Taulor G, Davies WJ (1991) Biophysics of leaf growth of hybrid poplar: impact of ozone. *New Phytol* **118**, 407-415.

Gebre GM, Kuhns MR, Brandle JR (1994) Organic solute accumulation and dehydration tolerance in three water-stressed *Populus deltoides* clones. *Tree Physiol.* **12**, 575-587.

Gebre GM, Tschaplinski TJ, Tuskan GA, Todd DE (1998) Clonal and seasonal differences in leaf osmotic potential and organic solutes of five hybrid poplar clones grown under field conditions. *Tree Physiol.* **18**, 645-652.

Giovannelli A, Deslauriers A, Fragnelli G, Scaletti L, Castro G, Rossi S, Crivellaron A (2007) Evaluation of drought response of two poplar clones (*Populusxcanadensis* Monch 'I-214' and *P. deltoides* Marsh. 'Dvina') through high resolution analysis of stem growth. *J Exp Bot* **58**, 2673-2683.

Griffin DH, Schaedle M, Manion PD, DeVit M (1991) Clonal variation in amino acid contents of roots, stems, and leaves of aspen (*Populus tremuloides* Michx.) as influenced by diurnal drought stress. *Tree Physiol.* **8**, 337-350.

Grossnickle SC (2000) Ecophysiology of Northern Spruce Species. The performance of planted seedling.. NRC Press - National Research Council Canada. Pp. 54-64

Gunasekera D, Berkowitz GA (1992) Evaluation of contrasting cellular-level acclimation responses to leaf water deficits in three wheat genotypes. *Plant Sci* **86**, 1-12.

Harayama H, Ikeda T, Ishida A, Yamamoto S (2006) Seasonal variations in water relations in current-year leaves of Evergreen trees with delayed greening. *Tree Physiol.* **26**, 1025-1033.

Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regul* **21**, 79-102.

Hinckley TM, Duhme F, Hinckley AR, Richter H (1980) Water relations of drought hardy shrubs: osmotic potential and stomatal reactivity. *Plant Cell Environ.* **3**, 131-140.

Kolowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to environmental stresses. *Bot Review* **68**, 270-334.

Kasuka M, Ohta M, Fujimura T (2005) Contribution of inorganic components to osmotic adjustment and leaf folding for drought tolerance in pearl millet. *Physiol Plant* **125**, 474-489.

Larson PR, Isebrands JG (1971) The Plastochron index as applied to developmental studies of cottonwood. *Can J For Res..***1**, 1-11.

Ladiges PY (1975) Some aspects of tissue water relations in three populations of *Eucalyptus viminalis* Labill. *New Phytol* **75**, 53-62.

Lei Y, Ying C, Li C (2006) Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of *Populus przewalksii. Physiol Plant* **127**, 182-191.

Marcum KB (2002) Growth and physiological adaptations of grasses to salinity stress. *In* M. Pessaraki (Ed.) Handbook of plant and crop physiology. Marcel Dekker, New York. Pp. 623–636.

Marron N, Villar M, Dreyer E, Delay D, Boudouresque E, Petit JM, Delmotte FM, Guehl M, Brignolas F (2005) Diversity of leaf traits related to productivity in 31 *Populus deltoides* × *Populus nigra* clones. *Tree Physiol.* **25**, 425–435.

Meloni DA, Gulotta MR, Martinez Ca, Oliva MC (2004) The effect of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba. Braz J Plant Physiol* **16**, 39-46.

Merchant A, Tausz M, Arndt SK, Adams MA (2006) Cyclitols and carbohydrates in leaves and roots of 13 Eucalyptus species suggest contrasting physiological responses to water deficit. *Plant Cell Environ.* **29**, 2017-2029.

Morgan JM (1984) Osmoregulation and water stress in higher plants. *Annual Rev Plant Physiol* **35**, 299-319.

Mullet JE, Whitsitt M (1996) Plant cellular responses to water deficit. *Plant Growth Regul* **20**, 119-124.

Nilsen ET, Orcutt DM (1996) Water limitation. *In*: Physiology of plants under stress. Abiotic factors. John Wiley and Sons (Eds). Pp. 322-361.

Niklas KJ (1992) Plant biomechanics: An engineering approach to plant form and function. Chicago. University of Chicago Press. Pp. 234-260.

Patakas A, Kidolaou N, Zioziou E, Radoglou K, Noitsakis B (2002) The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. *Plant Sci* **163**, 361-367.

Pregitzer KS, Friend AL (1996) The structure and function of Populus root systems, In: Stettler RF, Bradshaw HD, Heilman PE, Hinckley TM (Eds.), Biology of Populus and its implications for management and conservation. NRC Research press. Otawa. Pp. 331-354.

Proctor MCF, Nagy Z, Csintalan Z, Takács Z (1998) Water-content components in bryophytes: analysis of pressure-volume relationships. *J Exp Bot* **49**, 1845-1854.

Radin JW (1983) Physiological consequences of cellular water deficits: Osmotic adjustment, *In*: Taylor HM, Jordan WR, Sinclair TR (Eds.), Limitations to efficient water use in crop production. *Am Soc Agron.* Madison, WI. Pp. 227-265.

Ranieri A, Bernardi R, Lanese P, Soldatini GF (1989) Changes in free amino acid content and protein pattern of maize seedlings under water stress. *Env Exp Bot* **28**, 273-276.

Rascio A, Plantani C, Sealfati G, Tonti A, Di Fonzo N (1994) The accumulation of solutes and water binding strength in durum wheat. *Physiol Plant* **90**, 554–558

Roderick ML, Canny MJ (2005) A mechanical interpretation of pressure chamber measurements – what does the strength of the squeeze tell us? *Plant Physiol Bioch* **43**, 323-336.

Rose R, Rose CL, Omi SK, Forry KR, Durall DM, Bigg WL (1991) Starch determination by perchloric acid vs. enzymes: evaluating the accuracy and precision of six colorimetric methods. *J Agric Food Chem* **39**, 2-11.

Saito T, Soga K, Hoson T, Terashima I (2006) The bulk elastic modulus and the reversible properties of cell walls in developing *Quercus* leaves. *Plant Cell Physiol* **47**, 715-725.

SAS (2002) Version 9.1. SAS Institute, Cary NC US.

Sattelmacher B (2000) The apoplast and its significance for plant mineral nutrition. *New Phytol* **149**, 167-192.

Schobert B, Tschesche H (1978) Unusual solution properties of proline and its interactions with proteins. *Biochem Biophys Acta* **541**, 240–277.

Scholander PB. Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. *Science* **51**, 119-125.

Sivaramakrishnan S, Patell VZ, Flower DJ, Peacock JM (1988) Proline accumulation and nitrate reductase activity in contrasting sorghum lines during mid-season drought stress. *Physiol Plant* **74**, 418–426.

Smith D (1973) Influence of drying and storage conditions on non-structural carbohydrate analysis of herbage tissue – a review. *J Br Grassland Soc.* **28**, 129-134.

Stewart CR, Larher F (1980) Accumulation of amino acids and related compounds in relation to environmental stress. *In* The biochemistry of plants. Vol. 5. BJ Miflin (Ed.), Academic Press, New York. Pp. 609-635.

Stadelmann EJ (1984) The derivation of the cell wall elasticity function from the cell turgor potential. *J Exp Bot* **35**, 859-868.

Subbarao, EV, Nam NH, Chauhan YS, Johansen C (2000) Osmotic adjustment, water relations and carbohydrate remobilization in pigeonpea under water deficits. *J Plant Physiol* **157**, 651-659.

Tschaplinski TJ, Blake TJ (1989) Water stress tolerance and late-season organic solute accumulation in hybrid poplar. *Can J Bot* .**67**, 1681-1688.

Tschaplinski TJ, Tuskan GA (1994) Water-stress tolerance of black and eastern cottonwood clones and four hybrid progeny. II. Metabolites and inorganic ions that constitute osmotic adjustment. *Can J For Res***24**, 681-687.

Tschaplinski TJ, Tuskan GA, Gunderson CA (1994) Water-stress tolerance of black and eastern cottonwood clones and four hybrid progeny. I. Growth, water relations, and gas exchange. *Can J For Res***24**, 364-371.

Tschaplinski TJ, Tuskan GA, Gebre GM, Todd DE (1998) Drought resistance of two hybrid *Populus* clones grown in a large-scale plantation. *Tree Physiol.* **18**, 653-658.

Turner N C (1981) Techniques and experimental approaches for the measurement of plant water status. *Plant Soil* **58**, 339–366.

Turner NC, Jones MM (1980) Turgor maintenance by osmotic adjustment: A review and evaluation. *In*: Adaptation of plants to water and high temperature stress. Turner NC & Kramer PJ (Eds.). Stanford, California. USA.

Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *J Exp Bot* **23**, 267–282.

Tyree MT, Dainty J, Benis M (1972) The water relations of hemlock (*Tsuga Canadensis*): I. Some equilibrium water relations as measured by the pressure-bomb technique. *Can J Bot* .**51**, 1471-1480.

Tyree MT, Jarvis PG (1982) Water tissue and cells. *In* Physiological Plant Ecology II. Water Relations and Carbon Assimilation. Eds. O L Lange, P S Nobel, C B Osmond and H Zieglen. Encyclopedia of Plant Physiology New series, Volume 12 B. Springer-Vedag, Berlin. Pp 37-77

Yin C, Peng Y, Zang R, Zhu Y, Li C (2005) Adaptive responses of *Populus kangdingensis* to drought stress. *Physiol Plant* **123**, 445-451.

Yin C, Pang X, Lei Y (2009) Populus from high altitude has more efficient protective mechanisms under water stress than from low-altitude habitats: a study in greenhouse for cuttings. *Physiol Plant*. **137**, 22-35.

Zhang X, Zang R, Li C (2004) Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. *Plant Sci* **166**, 791-797.

Zimmermann U, Steudle E (1978) Physical aspects of water relations of plant cells. *Adv Bot Res* **6,** 45-117.

Watanabe S, Kojima K, Ide Y, Sasaki S (2000) Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica* in vitro. *Plant Cell Tiss Organ Cult* **63**, 199-206.

# Chapter Five
# Loss of root and stem hydraulic conductance and embolism recovery in poplar clones

## *5.1 Introduction*

Under drought conditions, adaptive strategies to maximize water uptake from the soil are important for plant survival. Water uptake is determined mainly by root hydraulic conductivity (*Lp*) which is composed of radial conductivity from the root surface to the root xylem and axial conductivity along the xylem (Steudle, 2000). Root hydraulic conductivity widely varies in plants and is affected by different factors including root anatomy, water availability and the properties of the xylem and root cells (Henzler *et al.*, 1999; Javot & Maurel 2000; Steudle 2000; Rincon *et al.*, 2003; Siemens & Zwiazek, 2004; Watanabe *et al.*, 2008). Poplars are generally described as highly productive at the expense of high water uptake (Tschaplinski *et al.*, 1994; Rood *et al.*, 2003). However, regardless of some degree of genetic diversity, poplars are known to be drought-sensitive (Rood *et al.*, 2000; Brignolas *et al.*, 2000; Dickmann *et al.*, 2001). The main reason for low tolerance to drought is that the xylem vessels of poplars cavitate at a relatively high water potential (begins around -1MPa) (Tyree *et al.*, 1992, 1994; Huckin *et al.*, 2005) Therefore, lowering *Lp* might help limit water loss by rapid early biophysical signalling inducing stomatal closure. Such a short- or long-term acclimation is crucial for plant survival when the soil is extremely dry. In addition, the capacity of root systems to recover *Lp* following drought stress is an important aspect of plant acclimation to water availability during dry summer periods (Brodribb, 2009).

Stem hydraulic conductance is an important characteristic of leaf water relations since the leaf water supply depends on hydraulic conductance. In the transpiration stream, water moves through the xylem and flows through the parenchyma cells before it evaporates through the stomata (Boyer, 1985; Steudle, 2000). However, the exact regulation mechanisms of hydraulic properties and their role in different drought resistance strategies of poplars remain unclear.

Xylem hydraulic conductivity, measured as the ratio of water flow rate and pressure gradient over a segment, has been positively correlated with water uptake and tree growth in trees (Vander Willigen & Pammenter, 1998). Xylem embolisms are considered to be among the main factors responsible for the decreased water conductance in drought-stressed poplars (Lo Gullo & Salleo, 1992; Hacke & Sauter 1995; Hacke & Sauter, 1996; Harvey & van den Driessche, 1997; Sparks & Black, 1999; Hukin *et al.*, 2005; Cochard *et al.*, 2007). The formation of xylem embolism is influenced by the anatomical and biophysical factors including vessel diameter, pit membrane structure, and surface tension of the xylem (Lovisolo *et al.*, 2008). Plants exposed to high levels of xylem embolism can experience complete loss of conductivity followed by death (Tyree & Sperry, 1988).

After significant embolism damage, embolized conduits may be able to transport water again by vessel refilling through the condensation of water vapour, dissolution of air, expulsion of air (Sperry *et al.*, 1987), and plant metabolism (Lovisolo *et al.*, 2008). Studies of root hydraulic conductivity in poplars are scarce, therefore, a better understanding of drought-induced embolism and refilling of the studied poplars is needed, since these clones exhibit different drought resistance responses, as demonstrated in the previous two chapters.

In this study, I examined stem xylem and root hydraulic conductance in several poplar clones exhibiting different drought resistance strategies, such as stomata closure, resistance to xylem cavitation, and leaf cell wall elasticity (Chapters 3 and 4). Given that these poplars exhibit different responses to drought, in this study, I tested whether root hydraulic conductivity is linked with drought-resistance mechanisms, and if these trees develop refilling mechanisms after drought relief. The main objectives of this study were to examine (1) the impact of different drought levels on transpiration and root hydraulic conductivity; (2) root hydraulic conductivity recovery following drought; (3) the degree of embolism and refilling of xylem vessels under different drought levels and following drought relief.

### *5.2 Materials and Methods*

Ten-cm-long stem cuttings of the following poplars were collected from the Alberta-Pacific Forest Industries Inc. (Al-Pac) experimental field site (54°49'N, 113°31'W), Alberta, Canada: P38P38 (*P. balsamifera* x *P. simonii* cv. P38P38), Walker (*P. deltoides* x (*P.* x *petrowskyana*) cv. Walker), Okanese (Walker x (*P. x petrowskyana*) cv. Okanese), Northwest [P. balsamifera x P. deltoides cv. Northwest (Jackii hybrid)], Assiniboine (open-pollinated Walker cv. Assiniboine), Berlin (*P. x berolinensis* cv. Berlin) and balsam poplar (*P. Balsamiferan* L.). The cuttings were transported to the University of Alberta where they were soaked in water at room temperature for two days prior to planting (DesRochers & Thomas, 2003). Each cutting was rooted in Metro Mix® 290 (Sun Gro Horticulture, Vancouver, BC, Canada $^{TM}$) growing medium in Styrofoam containers (440 ml volume/cell) (Beaver Plastic Ltd. AB, Canada) for 45 days and fertilized weekly with 200 mg $L^{-1}$ 15-30-15 (N-P-K) fertilizer solution (Plant Products Company Ltd., Brampton ON, Canada). Rooted cuttings were transferred to 4.5 L plastic pots filled with homogenized Metro Mix® 290 supplemented with 2.73 $g.L^{-1}$ Nutricote® 13-13-13 100 d slow-release fertilizer (6.5% $NO_3$-N, 6.5 $NH_4$-N, 13% $P_2O_5$, 13% $K_2O$, 1.2% Mg, 0.02% B, 0.05% Cu, 0.2% chelated Fe, 0.06% Mn, 0.02% Mo, 1.3% EDTA; Plant Products Company Ltd.). The plants were grown and watered daily in a greenhouse set to 22°C/18°C (day/night) temperatures, 20-35% relative humidity, and under natural light supplemented with incandescent lamps to maintain a 16-h photoperiod.

#### *5.2.1 Treatments*

Drought treatments were applied to plants approximately three months after rooting (plants approximately 1.7 m in height). The treatments were applied to two groups of plants of the same age. One group of plants was used for the measurements of transpiration and root hydraulic conductivity and the second group for stem conductivity determinations. All pots were saturated with water and allowed to dry until a desired level of soil water content (SWC) was achieved that had been experimentally determined to produce the symptoms of mild (≈

25% SWC) and severe (≈ 12% SWC) drought stress in plants. The symptoms of mild drought included a decrease in stomatal conductance and those of severe drought included leaf wilting and leaf necrosis with no plant mortality. The severe drought treatment was followed by drought recovery by rewatering the plants (SWC > 40%) for one day and two days. The control for each drought and recovery treatments, group consisted of well-watered plants (SWC > 40%). Some wilting of leaves was the only visible symptom of mild stress while severely-stressed plants showed high proportion of wilted leaves. Soil water content (SWC) was measured daily using a time-domain reflectometry (TDR) (Topp *et al.*, 1980).

### *5.2.2 Experimental design and measurements*

#### *5.2.2.1 Leaf transpiration and root hydraulic conductivity*

This experiment had a completely randomized block with five treatments (mild stress, severe stress, one-day recovery, two-day recovery, and well-watered control) and six poplar clones. Each treatment was applied to three trees per clone and replicated in two blocks. Blocking was in time, and the experiment was carried out during two weeks. On each day, a single treatment *versus* its control was measured on the six poplar clones.

For root hydraulic conductivity ($L_{pr}$) measurements, all of the above listed poplar clones were taken with the exception of Okanese. Stem xylem conductivity, root hydraulic conductivity, and transpiration rates ($E$) were measured for each treatment. Transpiration rates were measured in the abaxial surfaces of LPI 8 (Larson & Isebrands, 1971). The measurements were carried out at midday (11.00 – 13.00 h) using a steady-state porometer (LI-1600, Li-Cor Inc, Lincoln, NE, USA).

Root hydraulic conductance ($K_r$) was determined using the high pressure flow meter (HPFM) (Dynamax Inc., Houston, TX, USA) as described by Tyree *et al.* (1995). The stem was cut 2 cm above the soil surface while the whole shoot was held under water. Roots were connected to the HPFM system and pressurized to 0.4 MPa at a constant rate of 3-7 kPa $s^{-1}$ to obtain a pressure-flow

relationship. Hydraulic conductance (kg $MPa^{-1}$ $s^{-1}$) was then determined from the slope of the plot of flow versus the applied pressure. To determine root hydraulic conductivity ($L_{pr}$), root volume was measured by the water displacement method in a graduated cylinder (Voicu & Zwiazek, 2004) and $L_{pr}$ (kg $MPa^{-1}$ $s^{-1}$ $m^{-3}$) was then calculated by dividing $K_r$ by the root volume (Markhart & Smit, 1990).

#### *5.2.2.2 Xylem embolism and refilling*

This experiment used a completely randomized block consisting of seven poplar clones and four treatments (mild stress, severe stress, two-day recovery and well-watered control). Each treatment was applied to four trees per clone and the treatments were repeated with the second group of plants after one week. Each repeated experiment was considered a block and one clone for all treatments was measured per day.

Xylem conductivity was measured following the procedures described by Sperry *et al.* (1988). In the greenhouse, stems of 6 to 8 plants per clone per treatment were cut under water, wrapped immediately in plastic bags, to prevent drying, and taken to the laboratory. Stems were immersed in water and re-cut to obtain five segments of 2.5 cm in length. Hydraulic conductivity of each segment was then measured with a low pressure flow meter (LPFM). Stems were connected to the LPFM with Tygon tubing and sealed with Parafilm. The stem conductivity ($K_{stem}$) was measured by several transient measurements. Water flow into stem ($F$) and applied pressure ($P$) were measured every 5 s. Stem conductivity was then calculated as follows:

$$K_{stem} = \Delta F / \Delta P \qquad [9]$$

The degree of embolism was estimated as a percentage loss of conductivity with the maximum value (100% conductivity) obtained after removal of emboli (Sperry *et al.*, 1988; Cruiziat *et al.*, 2002). The initial conductivity ($K_i$, mol $MPa^{-1}$ m $s^{-1}$) was measured at gravity-induced force with 100 mM KCl solution at low flow pressure (3 kPa). Then, the samples were flushed for one minute at high pressure (120 kPa) with 100 mM KCl solution to remove emboli from conduits and obtain the maximum conductivity ($K_f$: mol $MPa^{-1}$ m $s^{-1}$).

Stem water potential ($\Psi_{stem}$) was measured using a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) (Scholander *et al.*, 1965). For $\Psi_{stem}$ measurements, LPI 10 was selected from each plant and was enclosed for 18 h in plastic bags and wrapped in aluminum foil to minimize transpiration water loss and create water potential equilibrium between the non-transpiring leaf and the stem.

### *5.2.3 Statistical analysis*

Analysis of variance (ANOVA) was performed using the *Proc Mixed* procedure of SAS (9.1) (SAS Institute, Cary NC US, 2002). The assumption of normality was assessed using the Proc UNIVARIATE function with the Kolmogorov-Smirnov test for normality. Drought and recovery treatment effects were analyzed using one way analysis of variance for each clone with block and block interaction terms considered random. A Kenward-Roger option was used to calculate denominator degrees of freedom. Least squared means were separated using pdiff option, and $p \leq 0.05$ was used to determine differences between treatments. Data were log-transformed for PLC and $\Psi_{stem}$ variables for the Assiniboine, balsam, Berlin and P38P38 clones. Root square transformation of $\Psi_{stem}$ for the Assiniboine clone was also used to satisfy assumptions of models. Linear models and ANOVA tables for each analysis are shown in Appendix E.

## *5.3 Results*

### *5.3.1 Transpiration rates (E) and root hydraulic conductivity ($L_{pr}$)*

Well-watered balsam poplar and Northwest clones showed the highest transpiration rates, whereas Walker and Berlin had the lowest rates. Overall, severe drought induced significant decreases in *E* for all clones. Transpiration rates decreased in severe-stressed plants of the Assiniboine clone ($p \leq 0.0001$) and P38P38 ($p=0.003$) compared with their controls (Fig 5-1 A and B). Northwest

clone showed significant decreases in *E* for water deficit (p=0.01 and p≤0.0001 for mild and severe respectively) and recovery treatments (p≤0.0001 and p=0.01 for recovery one and two days respectively). Balsam poplar decreased transpiration rates under severe drought (p≤0.0001) and recovery after one day (p=0.05) (Fig. 5-1D). Berlin and Walker clones significantly decreased *E* under both mild (p=0.006 and p=0.0008 respectively) and severe drought treatments (p≤0.0001 and p=0.02 respectively).

There were large differences in $L_{pr}$ between the well-watered plants of the studied clones with, the highest values $L_{pr}$ recorded for the Walker clone (Fig. 5-2F). Root hydraulic conductivity significantly decreased (34% *c.a.*) in Assiniboine under severe drought compared with its control (p=0.002). After one day of re-watering, $L_{pr}$ significantly increased in this clone (p=0.03). Root hydraulic conductivity showed similar values between control and treated plants for P38P38, with the exception of recovery after two days were $L_{pr}$ decreased in the treated plants (p=0.02) (Fig. 5-2A). Northwest and Berlin clones showed not differences between drought and recovery treatments compared with their controls (Fig. 5-2C and D). Balsam poplar showed small increases in $L_{pr}$ under mild stress and significantly higher for one day of re-watering (p=0.0002) compared with well watered plants (Fig. 5-2D). In general Walker clone showed higher $L_{pr}$ values for the drought and recovery treatments compared with their controls with significant increases under mild drought stress (p=0.01) (Fig. 5-2 F).

### *5.3.2 Effects of drought on stem conductivity*

Native embolisms were present in the studied poplar clones including almost 10% in well-watered controls (Fig. 5-3). In plants subjected to severe drought, percentage loss of conductivity (PLC) increased in all clones, but the differences between stressed and well-watered plants were statistically significant only for Northwest (p=0.0005) and balsam poplar (p=0.0003) (Figs. 5-3C and F). In Walker and Okanese, a small increase in PLC was observed in plants stressed with severe drought, and an increase of PLC was also observed when the plants were re-watered for 2 days (Figs. 5-3G and D). However, Assiniboine and P38P38 after stress relief showed high PLC and high error bars for these values (Fig. 5-3A and B).

The values of $\Psi_{stem}$ in plants subjected to the severe drought treatment, ranged from -0.7 MPa (15% PLC) for Okanese, -0.7 MPa (28% PLC) for P38P38, -0.9 MPa (13% PLC) for Walker, -1.0 MPa (17% PLC) for Assiniboine, -1.0 MPa (20% PLC) for Berlin, -1.2 MPa (30% PLC) for Northwest, to -1.6 MPa for balsam poplar (39% PLC) (Fig. 5-3). Significant differences between severe drought and control were found for P38P38 ($p<0.0001$), balsam poplar ($p<0.0001$), Northwest ($p<0.0001$), Walker ($p=0.0001$), Berlin ($p=0.007$), and Assiniboine ($p=0.008$). Re-watered plants reached the values of $\Psi_{stem}$ similar to those recorded in well-watered plants (Fig. 5-3).

### *5.4 Discussion*

Transpiration rates significantly decreased in response to mild drought stress treatment in Northwest, Berlin and Walker clones. This suggests that the main drought resistance strategy in these three poplar clones was to avoid water loss by reducing transpiration rates. The decrease in transpiration rates progressed with stress intensity for all treated poplar clones. Mild stress did not have a significant effect on $L_{pr}$ with the exception of Walker and balsam poplar where $L_{pr}$ increased as a result of mild drought. The increase in $L_{pr}$ could contribute to water loss avoidance (Tyree *et al.*, 2003). Increasing $L_{pr}$ could also delay stomatal closure, and help maintain stomatal conductance and hence photosynthesis during mild drought as reported in Chapter 3 for these two poplar clones (Figs. 3-3 and 3-4). An increase in $L_{pr}$ in response to mild drought was also reported in *P. tremuloides* Michx. (Siemens & Zwiazek, 2004).

In different poplar clones, $L_{pr}$ responded differently to the mild and severe drought treatment. During mild stress, Assiniboine and P38P38 and balsam poplar had similar or higher transpiration rates and $L_{pr}$, compared with controls; hence maintaining water movement from roots to leaves. Once these plants experienced severe drought, decreases in transpiration rates and root hydraulic conductivity were observed, possibly as a mechanism to prevent water loss from the root system. Reduction of $L_{pr}$ in Assiniboine might be used as a mechanism to avoid xylem embolism, since this clone is more sensitive to xylem embolism at relatively high stem water potentials (see Chapter 3, Fig. 3-8G). Decreases in $L_{pr}$

and transpiration might protect plants against xylem embolism when water is not available.

In addition to reduced stomatal conductance, severe drought often results in reductions of $L_{pr}$ (North & Nobel, 1992; Rieger, 1995; Siemens & Zwiazek, 2004), thus preventing loss of water from the roots to the soil by water potential differences. Slight or no decreases in $L_{pr}$ under drought for the other studied clones may reflect possible differences in root morphology and growth rates since older roots have suberised layers thus reducing water loss to the soil during drought stress (Taleisnik *et al.*, 1999; Vandeleur *et al.*, 2005). In addition, hydraulic resistance in these plants also increased due to severe water shortage in the soil, decreasing excessive water loss to the soil (Steudle, 2000).

After one day of rewatering, $L_{pr}$ increased almost two-fold in Assiniboine and balsam poplar. Since the response was rapid, it more likely involved changes in cell resistance to water flow than changes in root structure. Under these conditions, aquaporins could play an important role acting as valves and increasing $L_{pr}$ (Steudle, 2000). Aquaporin-mediated cell-to-cell water transport has been previously implicated in water flow regulation under drought stress (Vandeleur *et al.*, 2005, Zhang *et al.*, 2007; Galmés *et al.*, 2007). Aquaporins regulate water movement across cell membranes (Tyerman *et al.*, 2002) and have been involved in contributing to the decrease in $L_{pr}$ during drought conditions and the subsequent increase during re-watering (Javot & Maurel, 2002; Vandeleur *et al.*, 2005; Secchi *et al.*, 2007). In balsam poplar, increases of $L_{pr}$, after 1 day of re-watering, were accompanied by a reduction in transpiration. The capacity of the root system to recover hydraulic conductance following drought stress is an important feature of plant adaptation to seasonality in water availability, especially for these trees which undergo seasonal drought (Zhang *et al.*, 2001; Schindler & Donahue 2006).

Overall, in non-stressed plants, values of $L_{pr}$ were significantly different between poplar clones, and Walker plants had higher $L_{pr}$ than the remaining clones. High hydraulic conductivity of this poplar could probably be related to the larger diameter of the vessels. Small vessel diameter as well as high vessel density has been reported in poplar trees (Schume *et al.*, 2004). According to the Hagen-Poiseuille law, high transport capacity can be achieved by few vessels of larger diameter, leading to high transport efficiency per unit of cross-sectional

area, or by many vessels of small diameter, leading to high transport safety by pathway redundancy (Maseda & Fernández, 2006).

Increases in xylem tension due to soil water depletion induced several degrees of embolisms (PLC) in poplars; although no statistical differences were observed for most of the poplars under mild and severe drought. Northwest and balsam poplar clones showed significant loss of stem conductivity (*c.a.* 50%). For these two clones, xylem recovery was also observed after two days of re-watering. Variability within clones in the recovery response was also found, which could be possibly caused by different degrees of embolism induced by stress and when plants were re-watered probably not all of the vessels could be refilled.

Xylem recovery had been reported in other studies for poplars (Amlin & Rood, 2003) and other plants (Tyree *et al.*, 1999; McCully, 1999; Salleo *et al.*, 1996; Holbrook *et al.*, 2001; Salleo *et al.*, 2006; Lovisolo *et al.*, 2008). Several mechanisms of xylem refilling have been hypothesized including osmotic adjustment due to radial flow of sugars from depolymerisation of starch from the phloem (Salleo *et al.*, 2006), water movement from parenchyma cells to vessels due to aquaporin expression (Sakr *et al.*, 2003), dissolving of gas bubbles due to positive pressures in the embolized vessels (Konrad & Nebelsick 2003), activation of $Ca^{2+}$ channels inducing the activation of aquaporins (Netting, 2000), and an ABA residual signal that promotes gradual embolism repair (Lovisolo *et al.*, 2008). Aquaporins are important in regulating root hydraulic conductivity in *Populus* sp. (Wan & Zwiazek, 1999; Siemens & Zwiazek, 2003; Kohler *et al.*, 2003; Marjanović *et al.*, 2005). It is also possible that these proteins could be involved in drought-embolism recovery (Kaldenhoff *et al.*, 2008; Lovisolo *et al.*, 2008). These authors suggest that xylem recovery from drought involves the presence of aquaporins in the process of water loading from xylem surrounding parenchyma cells. However to determine whether any of these mechanisms contributes to refilling xylem vessels in poplars needs more investigation.

In conclusion, the present results confirm combined mechanisms of drought resistance in the studied poplars, described also in previous chapters (Tables 3-1, 4-5 and 5-1). Two of the most prominent poplar clones displaying these combined mechanisms were Walker and balsam poplar. Walker clone maintained relatively high root hydraulic conductivity and is moderately resistant to xylem cavitation, hence allowing water uptake by roots under severe drought.

This clone also showed accumulation of compatible solutes in leaves and roots, maintaining cell turgor and water balance in the plant. Conversely, balsam poplar decreased transpiration and root hydraulic conductivity under severe drought, therefore avoiding water loss from roots. This clone is also less vulnerable to xylem cavitation.

In addition, the range of $\Psi_{stem}$ variable under induced-drought conditions, using both Cavitron technique and withholding water in the greenhouse, showed similar values (see Chapter 3). Even though, induced-embolisms in the greenhouse were lower than using the Cavitron, these results corroborates that using the Cavitron could be useful as a screening technique for identifying poplars with drought resistance characteristics.

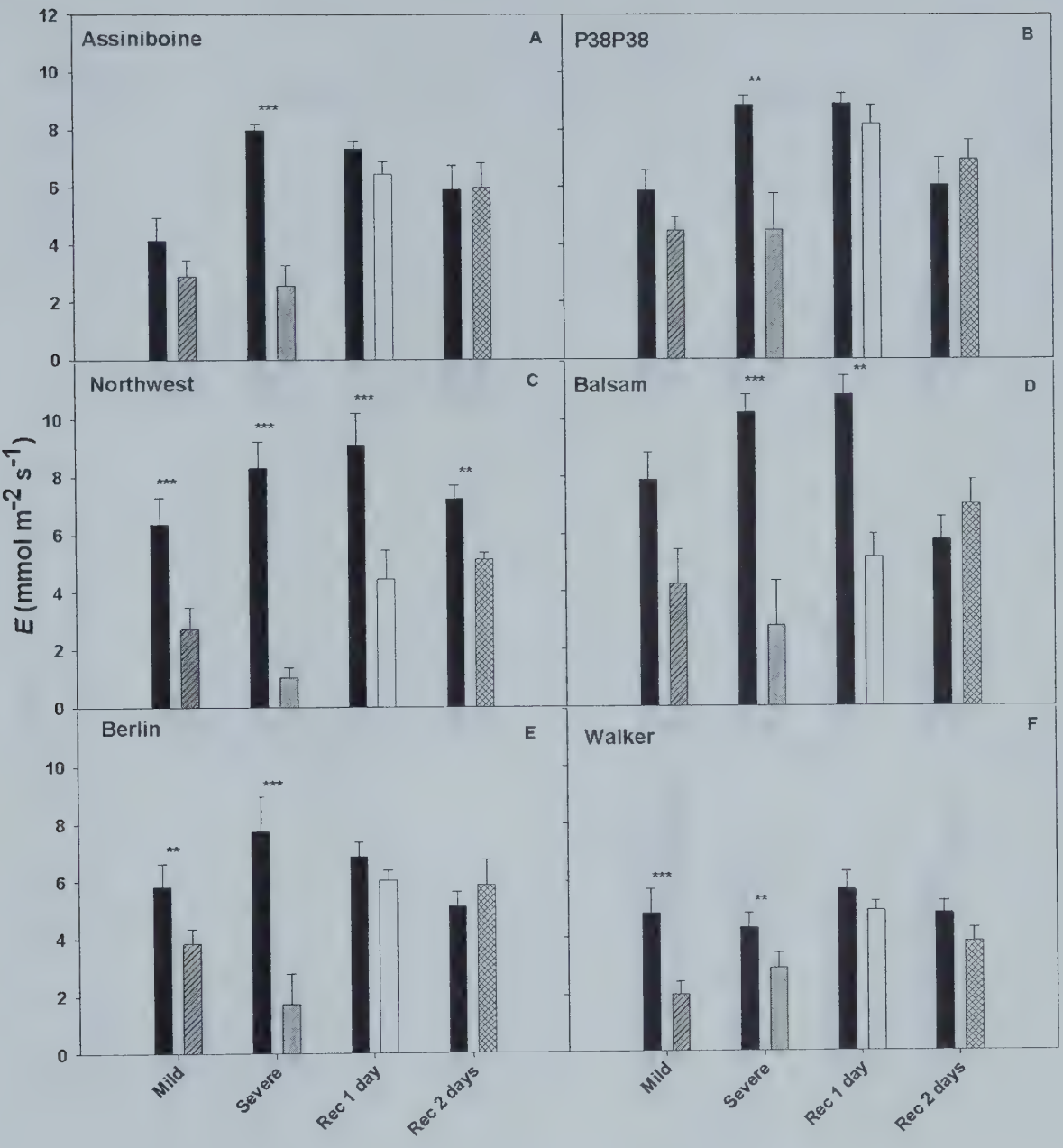



**Figure 5-1.** Transpiration (*E*) (means + SE, n=5-6) of poplar clones subjected to different soil water treatments (mild, severe, recovery 1 days and recovery two days). (A) Assiniboine, (B) P38P38, (C) Northwest (D) Balsam, (E) Berlin and (F) Walker. Asterisk corresponds to statistically significant differences between treatments for each clone compared with its control; differences at $p \leq 0.05$ (**) and $p \leq 0.001$ (***).

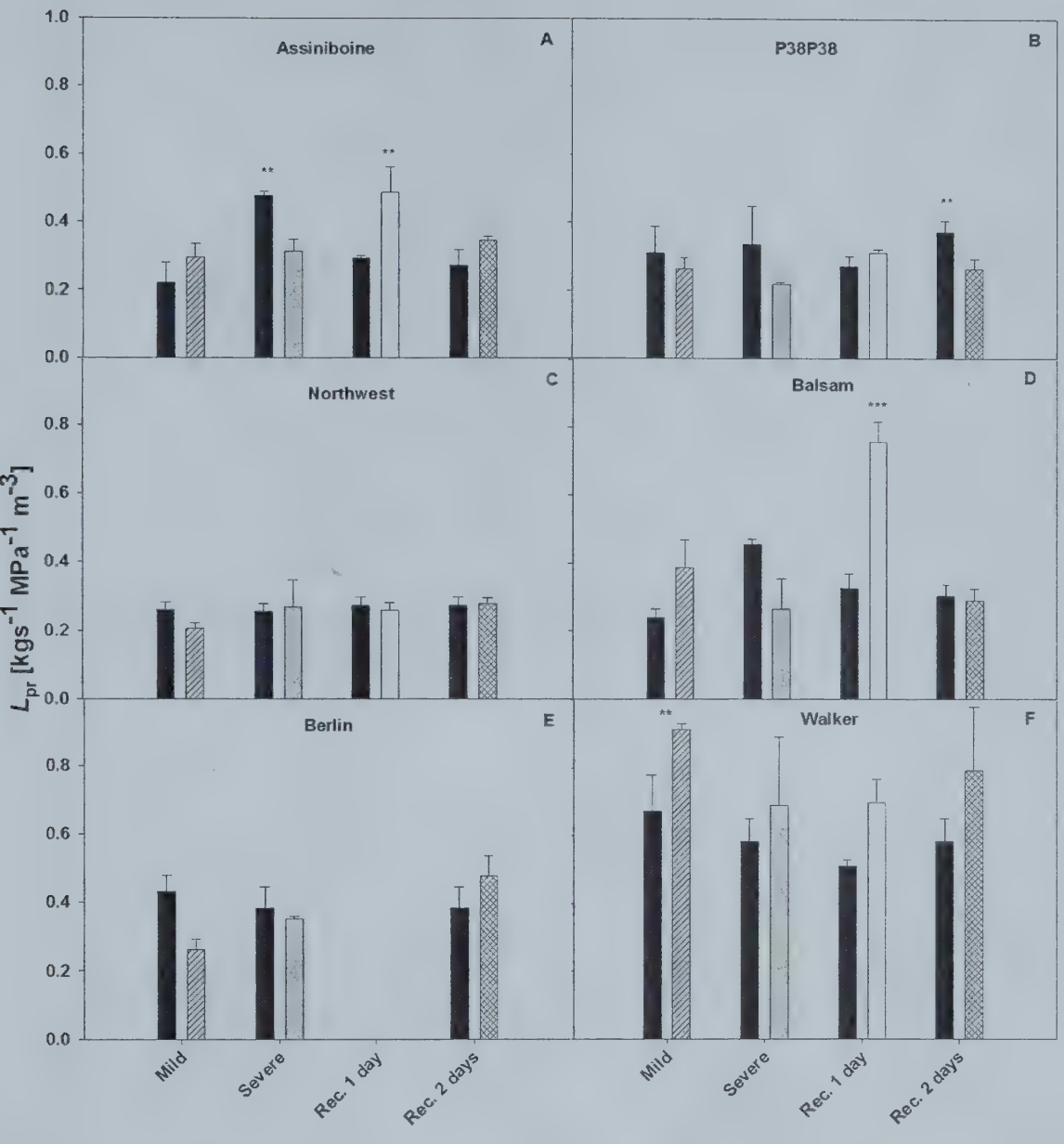



**Figure 5-2.** Root hydraulic conductivity ($L_{pr}$) (means + SE, n=5-6) of poplar clones subjected to different soil water treatments (mild, severe, recovery 1 days and recovery two days). (A) Assiniboine, (B) P38P38, (C) Northwest (D) Balsam, (E) Berlin and (F) Walker. Asterisk corresponds to statistically significant differences between treatments for each clone compared with its control; differences at $p \leq 0.05$ (**) and $p \leq 0.001$ (***).

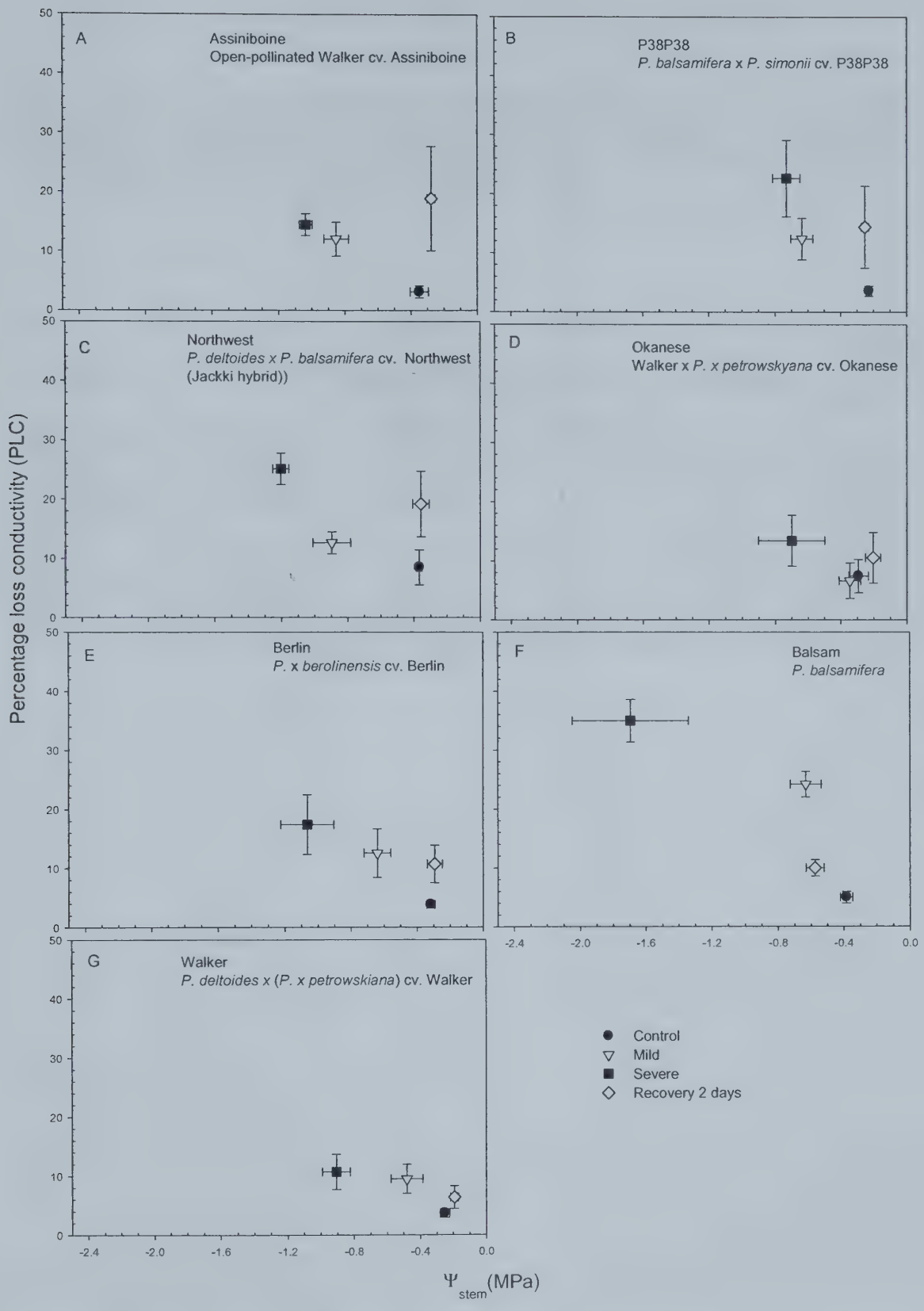



**Figure 5-3.** Relationship between percentage loss of conductivity (PLC) to a decrease in xylem water potential ($\Psi_{stem}$) along main stems of rooted cuttings for seven poplar clones (means ± SE, n=8). (A) Assiniboine, (B) P38P38, (C) Northwest (D) Okanese, (E) Berlin, (F) Balsam, and (G) Walker.

**Table 5-1.** Summary of the physiological responses of the poplar clones under severe drought stress. Putative drought resistance classification was based on the combination of responses showed in this study and chapters 3 and 4. (↓) Decrease, (↑) increase or (↔) no significant changes compared with control plants

| Poplar clone | Transpiration (*E*) | Root hydraulic conductivity ($L_{pr}$) | Stem water potential ($\Psi_{stem}$) | Loss of conductivity | Putative drought resistance |
|---|---|---|---|---|---|
| **Assiniboine** | 68% (↓) | 35% (↓) | -1.0 | 17% | Low |
| **P38P38** | 49% (↓) | (↔) | -0.73 | 28% | Moderate |
| **Northwest** | 88% (↓) | (↔) | -1.21 | 30% | Moderate |
| **Okanese** | | | -0.7 | 16% | Moderate |
| **Berlin** | 73% (↓) | (↔) | -1.06 | 20% | Low |
| **Balsam** | 78% (↓) | (↔) | -1.7 | 40% | Moderate |
| **Walker** | 33% (↓) | 15% (↑) | -0.91 | 13% | Moderate |

* Estimated percentage of decrease or increase compared with control plants

## *References*


Amlin NM, Rood SB (2003) Drought stress and recovery of riparian cottonwoods due to water table alteration along Willow Creek, Alberta. *Trees.* **17**, 351-358.

Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Ann For Sci.* **63**, 625-644.

Boyer JS (1985) Water transport. *Annu Rev Plant Physiol.* **36**, 473–516.

Brignolas F, Thierry C, Guerrier G, Boudouresque E (2000) Compared water deficit response of two *Populus euramericana* clones, Luisa Avanzo and Dorskamp. *Ann For. Sci.* **57**, 261–266.

Brodribb TJ (2009) Xylem hydraulic physiology: The functional backbone of terrestrial plant productivity. *Plant Sci.* **177**, 245-251.

Cochard H, Casella E, Mencuccini M (2007) Xylem vulnerability to cavitation varies among poplars and willow clones and correlates with yield. *Tree Physiol.* **27**, 1761-1767,

Comstock JP (2002) Hydraulic and chemical signaling in the control of stomatal conductance and transpiration. *J Exp Bot* **53**, 195-200.

Cruiziat P, H Cochard, Améglio T (2002) Hydraulic architecture of trees: main concepts and results. *Ann For Sci.* **59**, 723-752.

Dickmann DI (2001) An overview of the genus Populus. *In:* Poplar culture in North America. Part A, Chapter 1. Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J (Eds). NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada. Pp. 1-42.

Galmés J, Pou A, Alsina MM, Tomàs M, Medrano H, Flexas J (2007) Aquaporin expression in response to different water stress intensities and recovery in Richter-110 (*Vitis* sp.): relationship with ecophysiological status. *Planta.* **226**, 761-681.

Hacke U, Sauter JJ (1995) Vulnerability of xylem to embolism in relation to leaf water potential and stomatal conductance in *Fagus sylvatica f. purpurea* and *Populus balsamifera*. *J Exp Bot* **46**, 1177-1183.

Hacke U, Sauter JJ (1996) Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. *Plant Physiol.* **111**, 413-417.

Harvey HP, Van den Driessche R (1997) Nutrition, xylem cavitation and drought resistance in hybrid poplar. *Tree Physiol.* 17, 647-654.

Henzler T, Waterhouse RN, Smyth AU, Carvajal M, Cooke DT, Schäffner AR, Steudle E, Clarkson DT (1999) Diurnal variations in hydraulic conductivity and root pressure can be correlated with the expression of putative aquaporins in the roots of *Lotus japonicus*. *Planta* **210**, 50-60.

Hsiao TC, Acevedo E, Gereres E, Henderson DW (1976) Stress metabolism: water stress, growth and osmotic adjustment. *Phil Trans R Soc Lond B.* **273**, 479-500.

Holbrook NM, Ahrens ET, Burns MJ, Zwieniecki MA (2001) In vivo observation of cavitation and embolism repair using magnetic resonance imaging. *Plant Physiol.* **126**, 27-31.

Hukin D, Cochard H, Dreyer E, Le Thiec D, Bogeat-Triboulot MB (2005) Cavitation vulnerability in roots and shoots: does *Populus euphratica* Oliv., a poplar from arid areas of Central Asia, differ from other poplar species? *J Exp Bot* **56**, 2003-2010.

Javot H, Maurel C (2002) The role of aquaporins in root water uptake. *Ann Bot.* **90**, 301-313.

Jones HG. Corleyy JE (1992). Current topics in drought physiology. *J Agric Sci.* **119**, 291-6.

Kaldenhoff R, Ribas-Carbo M, Sans JF, Lovisolo C, Heckwolf M, Uehlein N (2008) Aquaporins and plant water balance. *Plant Cell Environ.* **31**, 658-666.

Kohler A, Delaruelle C, Martin D, Encelot N, Martin¯ F (2003) The poplar root transcriptome : analysis of 7000 expressed sequence tags. *FEBS* **542**, 37-41.

Konrad W, Roth-Nebelsick A (2003) The dynamics of gas bubbles in conduits of vascular plants and implications for embolism repair. *J Theor Biol.* **224**, 43-61.

Lo Gullo MA, Salleo S (1992) Water shortage in the wood and xylem cavitation in 1-year-old twigs of *Populus deltoides* Bartr. *Plant Cell Environ.* **15**, 431-438.

Lovisolo C, Perrone I, Hartung W, Schubert A (2008) An abscisic acid-related reduced transpiration promotes gradual embolism repair when grapevines are rehydrated after drought. *New Phytol* **180**, 642–651

Luu DT, Maurel C (2005) Aquaporins in a challenging environment: molecular gears for adjusting plant water status. *Plant Cell Environ.* **28**, 85–96

Marjanović Z, Uehlein N, Kaldenhoff R, Zwiazek JJ, Weiß M, Hampp R, Nehls U (2005) Aquaporins in poplar: what a difference a symbiont makes! *Planta.* **222**, 258-268.

Markhart AH, Smit B (1990) Measurement of root hydraulic conductance. *Hortic. Sci.* **25**, 282–287.

McCully ME (1999) Root xylem embolisms and refilling. Relation to water potentials of soil, roots, and leaves, and osmotic potential of root xylem sap. *Plant Physiol.* **119**, 1001-1008.

Muhsin TM, Zwiazek JJ (2002) Ectomycorrhizas increase apoplastic water transport and root hydraulic conductivity in *Ulmus americana* seedlings. *New Phytol* **153**, 153-158.

Netting AG (2000) pH, abscisic acid and the integration of metabolism in plants under stressed and non-stressed conditions: cellular responses to stress and their implication for plant water relations. *J Exp Bot* **51**, 147-158.

North GB, Nobel PS (1992) Drought-induced changes in hydraulic conductivity and structure in roots of *Ferocactus acanthodes* and *Opuntia ficus-indica*. *New Phytol* **120**, 9-19.

Rieger M (1995) Offsetting effects of reduced root hydraulic conductivity and osmotic adjustment following drought. *Tree Physiol.* **15**, 379-385.

Rincon CA, Raper Jr. CD, Patterson RP (2003) Genotypic differences in root anatomy affecting water movement through roots of soybean. *Int J Plant Sci* **164**, 543-551

Rood SB, Patino S, Coombs K, Tyree MT (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees.* **14**, 248-257.

Rood SB, Braatne JH, Hughes FMR (2003) Ecophysiology of riparian cottonwoods: Stream flow dependency, water relations and restoration. *Tree Physiol.* **23**, 1113–1124.

Sakr S, Alves G, Morillon R, Maurel K, Decourteix M, Guilliot A, Fleurat-Lessard P, Julien JL, Chrispeels MJ (2003) Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiol.* **133**, 630-641.

Salleo S, Lo Gullo MA, de Paoli D, Zippo M (1996) Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. *New Phytol* **132**, 47-56.

Salleo S, Trifilò P, Lo Gullo MA (2006) Phloem as a possible major determinant of rapid cavitation reversal in stems of *Laurus nobilis* (laurel). *Funct Plant Biol.* **33**, 1063-1074.

SAS (2002) Version 9.1. SAS Institute, Cary NC US.

Schindler DW, Donahue WF (2006) An impeding water crisis in Canada's western prairie provinces. *PNAS* **103**, 7210-7216.

Schume H, Grabner M, Eckmüllner O (2004) The influence of an altered groundwater regime on vessel properties of hybrid poplar. *Trees.* **18**, 184-194.

Secchi F, Lovisolo C, Uehlein N, Kaldenhoff R, Schubert A (2007) Isolation and functional characterization of three aquaporins from olive (*Olea europaea* L.). *Planta.* **225**, 381-392.

Siemens JA, Zwiazek JJ (2004) Changes in root flow properties of solution culture-grown trembling aspen (*Populus tremuloides*) seedlings under different intensities of water-deficit stress. *Physiol Plant* **121**, 44-49.

Sparks JP, Black RA (1999) Regulation of water loss in populations of *Populus trichocarpa:* the role of stomatal control in preventing xylem cavitation. *Tree Physiol.* **19**, 453-459.

Sperry JS, Holbrook MN, Zimmermann MH, Tyree MT (1987) Spring filling of xylem vessels in wild grapevine. *Plant Physiol.* **83**, 414-417.

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Env.* **11**, 35-40.

Sperry JS (1986) Relationship of xylem embolism to xylem pressure potential, stomatal closure, and shoot morphology in the palm *Rhapis excelsa*. *Plant Physiol.* **80**, 110-116.

Stedule E (2000) Water uptake by roots: Effects of water deficit. *J Exp Bot* **51**, 1531-1542.

Steudle E, Ramatunghe K (2007). Apoplastic water transport in roots. *In*: The apoplast of higher plants: compartment of storage, transport and reactions. The significance of the apoplast for the mineral nutrition of higher plants. Sattelmacher B, Horst WJ (Eds). Springer Verlag. Dordrecht, The Netherlands. Pp. 119-130.

Stout DL, Sala A (2003) Xylem vulnerability to cavitation in *Pseudotsuga menziesii* and *Pinus ponderosa* from contrasting habitats. *Tree Physiol.* **23**, 43-50.

Taleisnik E, Peyrano G, Cordoba A, Arias C (1999) Water retention capacity in root segments differing in the degree of exodermis development. *Ann Bot*. **83**, 19-27.

Tschaplinski TJ, Tuskan GA, Gunderson CA (1994) Water-stress tolerance of black and eastern cottonwood clones and four hybrid progeny. 1. Growth, water relations, and gas exchange. *Can J For Res***24**, 364–371.

Tsuda M, Tyree MT (2000) Plant hydraulic conductance measured by the high pressure flow meter in crop plants. *J Exp Bot* **51**, 823-828.

Tyerman SD, Niemietz CM, Bramley H. (2002) Plant aquaporins: multifunctional water and solute channels with expanding roles. *Plant Cell Environ.* **25**, 173–194.

Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiol*. **88**, 574-580.

Tyree MT, Alexander J, Machado JL (1992) Loss of hydraulic conductivity due to water stress in intact juveniles of *Quercus rubra* and *Populus deltoides*. *Tree Physiol.* **10**, 411-415.

Tyree MT, Kolb KJ, Rood SB, Patiño S (1994) Vulnerability to drought-induced cavitation of riparian cottonwoods in Alberta: a possible factor in the decline of the ecosystem? *Tree Physiol.* **14**, 455-466.

Tyree MT, Patiño S, Bennink J, Alexander J (1995) Dynamic measurements of roots hydraulic conductance using a high-pressure flowmeter in the laboratory and field. *J Exp Bot* **46**, 83-94.

Tyree MT, Salleo S, Nardini A, Lo Gullo MA, Mosca R (1999) Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? *Plant Physiol.* **120**, 11-21.

Tyree MT (2003) Hydraulic limits on tree performance: transpiration, carbon gain and growth of trees. *Trees*. **17**, 95-100.

Vander Willigen C, Pammenter NW (1998) Relationship between growth and xylem hydraulic characteristics of clones of *Eucalyptus* spp. at contrasting sites. *Tree Physiol.* **18**, 595-600.

Vandeleur R, Niemietz C, Tilbrook J, Tyerman SD (2005) Roles of aquaporins in root responses to irrigation. *Plant Soil* **274**, 141-161.

Voicu MC, Zwiazek JJ (2004) Cyclohexamide inhibits root water flow and stomatal conductance in aspen (*Populus tremuloides*) seedlings. *Plant Cell Environ.* **27**, 199-208.

Wan X, Zwiazek JJ (1999) Mercuric chloride effects on root water transport in aspen seddlings. *Plant Physiol* **121**, 939-946.

Wan X, Zwiazek JJ (2001) Root water flow and leaf stomatal conductance in aspen (*Populus tremuloides*) seedlings treated with abscisic acid. *Planta*. **213**, 741-747.

Wasilewski A, Vla F, Sirichandra C, Redko Y, Jammes F, Valon C, Frei dit Frey N, Leung J (2008) An update on Abscisic Acid signalling in plants and more. *Mol Plant.* **1**, 198-217.

Watanabe Y, Tobita H, Kitao M, Maruyama Y, Choi D, Sasa K, Funada R, Koike T (2008) Effects of elevated $CO_2$ and nitrogen on wood structure related to water transport in seedlings of deciduous broad-leaved tree species. *Trees.* **22**, 413-411.

Weatherley PE (1982) Water uptake and flow in roots. In: Physiological Plant Ecology II. Water relations and carbon assimilation. (Lange OL, Nobel PS, Osmond CB, Ziegler H eds). Springer-Verlag Berlin Heidelberg New York. Pp 79-109.

Zhang X, Harvey KD, Hogg WD, Yuzyk TR. (2001) Trends in Canadian Streamflow, *Water Resour Res* **37**, 987–998.

Zhang Y, Wang Y, Jiang L, Xy Y, Wang Y, Lu D, Chen F (2007) Aquaporin JcPIP2 is involved in drought responses in *Jatropha curcas*. *Acta Biochim Biophys Sin.* **39**, 787-794.

# Chapter Six
# Synthesis

## *6.1 Summary*

This research had the objectives of providing a basis for the establishment of an informative screening technique to identify traits related to drought resistance and to gain a better understanding of the drought resistance mechanisms in balsam and hybrid poplars from Sections *Aigerios* and *Tacamahaca* (*Populus* spp.). *Populus* was chosen in this study because of its ecological and commercial importance. These trees have also been tested in Al-Pac's research trials for a wide range of drought stress responses (DesRochers *et al.*, 2007). Because drought resistance involves several mechanisms in plants, which cannot be considered independent, the integration and timing of these mechanisms are fundamental for the regulation of water transport, leaf turgor and drought resistance. Therefore, this research is considered to be important for several reasons:

(1) Poplars used in this study are economically important for poplar-farming programs in western Canada.

(2) Several studies have documented selected drought resistance mechanisms in poplars (Tschaplinski *et al.*, 1998; Rood *et al.*, 2000; Yin *et al.*, 2005; Cochard *et al.*, 2007), however, there is little information on the integrated mechanisms used by these plants to resist drought at the whole plant level.

(3) Although there are few studies identifying the role of compatible solutes in drought resistance in poplars (Tschaplinski & Blake 1989; Yin *et al.*, 2005), there is a lack of detail about the involvement of osmoregulators in root responses under severe drought and recovery after re-watering treatments.

(4) Vulnerability to xylem cavitation and stomatal regulation have been involved as the two main characteristics to drought

resistance in several species (Sperry *et al.*, 1998; Linton *et al.*, 1998; Sparks & Black 1999). However, there is little information about stomatal regulation in relation to xylem vulnerability to cavitation in poplars. It is important to understand how the integrated mechanisms of these poplars withstand different drought levels that they are continually experiencing under field conditions.

(5) In addition to stomatal conductance and stem conductance, root hydraulic conductance has been reported to play a central role in regulating water relations and tree growth (Wan & Zwiazek, 1999; Steudle, 2002). However, studies combining root hydraulic conductance, transpiration and xylem conductance during drought and recovery after severe drought are scarce. In my study, a better understanding of the involvement in root hydraulic conductivity in response to drought and recovery was gained.

(6) In general, my study highlights several aspects for a potential screening protocol to evaluate poplar clone resistance to drought, when being considered for use in the prairies.

The research presented in this dissertation focused on evaluating drought resistance characteristics related to root hydraulic conductance, stomatal regulation, stomatal characteristics, and leaf and root osmoregulation and stem xylem cavitation responses under different drought levels and recoveries (outlined in Chapter 1). Chapter 3 discussed the first two objectives of how stomatal regulation, stomatal characteristics and xylem vulnerability to cavitation are involved in drought resistance traits. Chapter 4 followed with the evaluation of cell turgor and osmoregulation in leaves and roots as water is depleted, and the understanding of these compatible solutes in the process of drought recovery in poplars. Chapter 5 determined the role of root hydraulic conductivity in the process of adjustment drought stress, and the degree of stem xylem refilling after the rewatering process in poplars.

The results showed that the physiological bases for drought-resistance varies in different poplar clones; and also that there are parallel changes in the physiological and biochemical responses when these trees are exposed to

drought and subsequent recovery conditions. In addition to this, the development of soil retention curves for the Metro Mix ® substrate (Appendix C) gave accurate measurements of soil water potential, and thus a better tool to understand the water retention capacity of this type of substrate. This information will be a useful base for further studies that use Metro Mix ® soil as a growing substrate.

### *6.2 Mechanisms related to drought resistance*

#### *6.2.1 Stomata regulation*

Stomatal characteristics have been shown to be involved in drought-resistance (Bond & Kavanagh, 1999; Cochard *et al.*, 2002) and metabolism efficiency (Nilsen & Orcutt, 1996). In this study, stomatal characteristics such as large size and density were not related to stomatal conductance as reported for other poplars (Pearce *et al.*, 2006). Nevertheless, small size of guard cells for Walker could be responsible for the turgor maintenance, induction of stomata to remain open (Spence *et al.*, 1986), and subsequent carbon gain through photosynthesis under severe drought conditions.

Stomatal regulation by opening or closing the stomata under severe drought was closely related to xylem vulnerability characteristics and the responsive ability of the plant to withstand drought stress. For example, Northwest and balsam poplar had greater stomatal opening under severe drought, but these clones were less sensitive to xylem cavitation. Accordingly, an induction of leaf necrosis and shedding of mature leaves was observed, thus providing a reduction in the transpiration demand for these clones. Walker also maintained relatively open stomata under drought, but conversely this clone had better cell turgor control. Closure of stomata for Okanese and Assiniboine may have contributed to plant water conservation since these plants were more sensitive to xylem cavitation. Stomata closure in P38P38 could be a conservative mechanism, avoiding loss of water through the leaves, since it also showed a relatively low vulnerability to xylem cavitation. The efficiency of stomatal control of water loss is an important factor which must be taken into account when

determining the probability of xylem cavitation in stressed plants (Sperry *et al.*, 1993; Ennajeh *et al.*, 2008).

In summary, stomatal characteristics were probably not related to drought resistance mechanisms for these poplars. On the other hand, stomatal regulation, by opening or closing the stomata, was closely related to drought-resistance characteristics and also to the xylem vulnerability to embolism.

### *6.2.2 Xylem vulnerability to embolism*

A serious consequence of xylem embolism is the loss of functional xylem. Thus, the adaptation mechanism to vulnerability to xylem cavitation is important to conserve water movement and minimise detrimental consequences of drought. Two drought-resistance strategies were found for different poplars under soil water depletion: cavitation avoidance and tolerance. Both P38P38 and Okanese relied on stomatal closure to maintain high $\Psi_{stem}$ values given that their xylems were relatively vulnerable to cavitation, indicating an avoidance strategy. The noticeable tolerance strategy in Northwest, balsam poplar and Walker, on the other hand, allowed these plants to maintain water movement through the plant system by maintaining open stomata. The Berlin clone showed poor stomatal control in addition to early cavitation with low xylem water potentials, therefore, this clone was considered relatively sensitive to cavitation. Lastly, Assiniboine showed a peculiar, double s-shape curve, response to cavitation, suggesting that xylem architecture played an important role in this response.

In conclusion, a functional coordination by a timed response of the stomata to reduced hydraulic conductivity was most likely the main factor associated with preventing extensive loss of vessel conductivity due to induced cavitation in these poplars. A notable insight that emerges from these results is that although stomata regulation was involved in resistance to xylem cavitation, xylem architecture could also be playing an important role. However, in the whole plant system, interactions between hydraulic conductivity, stomatal regulation and xylem architecture make interpretations complicated.

### *6.2.3 Osmotic adjustment*

At the cellular level, osmotic adjustment and accumulation of compatible solutes play an important role in lowering the osmotic potential, thereby contributing to the maintenance of turgor as water potential decreases (Turner & Jones, 1980; Verslues *et al.*, 2006). The properties of cell walls also play an important role in drought resistance (Verslues *et al.*, 2006).

### *6.2.4 Carbohydrates and amino acids*

Accumulation of organic solutes did not appear to play a critical role in drought resistance mechanisms as observed by Tschaplinski & Blake (1989) in other poplar hybrids. However, in this study the accumulation of total amino acids, proline and starch played a role after rewatering for Walker and P38P38 poplar clones. As drought developed, only Walker poplar showed osmotic adjustment by increasing proline accumulation in leaves and roots. This could contribute to maintaining water uptake and also protecting the subcellular structure from damage (Schobert & Tschesche, 1978). On the other hand, increases in starch accumulation in leaves of P38P38 poplar facilitated the use of available reserves for the plant.

In this study, no indications of osmotic adjustments in leaves, due to carbohydrate and amino acid accumulation, were observed for most of the poplar clones under drought stress. However, a recovery capacity was found, by the increase of accumulation of reserves of carbon and nitrogen (in the form of proline), in Assiniboine, Northwest and balsam poplars.

### *6.2.5 Cell properties*

Cell wall properties did not differ between poplar clones. Elastic bulk modulus and symplastic values were higher in Northwest, compared with the rest of the clones, indicating that cell walls are thinner and have a better capacity to maintain turgor during drought stress; as revealed when these plants experienced severe drought and maintenance of open stomata was observed

(Chapter 3). The lower symplastic fraction and elastic bulk modulus values for the rest of the clones, indicates more capacity of organic or inorganic solutes accumulation, as hypothesized by Nilsen & Orcutt (1996). This accumulation was revealed in Walker and P38P38 clones (Chapter 4).

### *6.2.6 Root hydraulic adjustments*

Adjustments in roots by decreasing or increasing hydraulic conductivity ($L_{pr}$) were observed in the tested poplar clones. Reduction of $L_{pr}$ maintained water potential below the threshold for cavitation under severe drought in Assiniboine, P38P38 and balsam poplar. Conversely, increases of $L_{pr}$ acted as an adaptive mechanism to maximize water transfer and consequently carbon fixation in the Walker clone. Slight or no decreases in $L_{pr}$ for the other clones, under severe drought, was probably due to roots in woody species having suberised layers may reduce water loss to the soil during drought stress (Taleisnik *et al.*, 1999; Vandeleur *et al.*, 2005). Hydraulic resistance of these plants increased and water flow through the plant was thus maintained.

Root hydraulic increases were largely observed after re-watering treatments indicating that poplar root systems have the capacity to recover after drought. This capacity to recover could be an indication of rapid radial flow of water along the apoplast and a parallel cell-to-cell path by the aquaporins expression, as reported by Vandeleuar *et al.* (2005). The capacity of the root system to recover hydraulic conductance following drought stress is an important feature of plant adaptation to seasonality in water availability, especially for these temperate trees which typically undergo seasonal drought.

Overall, $L_{pr}$ played an important role in adjusting water transport, from the soil to the roots, in combination with other plant modifications (*i.e.* stomata adjustments or solutes accumulation capacity in roots). Most importantly, root hydraulic conductivity was modified after plant re-watering. It can be considered an important mechanism for drought recovery in these trees. Lastly, hydraulic conductivity was significantly different among poplars; whether these differences were due to their efficiency of water-transport system, or to different levels of drought stress experienced by each poplar are still unclear.

### *6.2.7 Refilling mechanism in xylem stems*

The re-watering treatments restored water transport in most of the vessels that had embolized during the drought stress treatments. According to Salleo *et al.* (2004), refilling mechanisms involve osmotic adjustments and phloem-driven xylem refilling. Based on the accumulation of some solutes in roots and leaves of P38P38 and Walker (Chapter 4), it can be proposed that some osmolytes contributed to vessel refilling in these two clones. However, because of the high variability of poplar responses under drought, it is difficult to elucidate which process is contributing to embolism repair for the rest of the evaluated clones. Thus, further studies are needed to understand how the process of embolism repair occurs in poplar clones.

The rapid recovery (after 30 minutes of re-watering) in poplars has not been reported previously. Regardless of the mechanism(s) responsible for vessel refilling, the ability of poplar clones to rapidly repair cavitated xylem conduits (Chapter 5) has important implications for understanding regulation of water movement in poplars.

## *6.3 Hypothesized mechanisms of drought resistance*

The phenomenon of drought is complex due to several interactions between precipitation, evapotranspiration, irradiation, nutrition availability and soil characteristics. Therefore, drought resistance mechanisms are also complex and are the result of physiological, biochemical and structural factors, and the intensity and duration of water deficit. These factors might be adjusted to either tolerate or avoid drought conditions. However, it is difficult to clearly separate tolerant and avoidant plants since the interaction of these factors and the timing response play an important role in the drought resistance mechanisms. In this study, characterization of the studied poplars considered the interaction of several physiological responses as illustrated in Figure 6-1.

### *6.3.1Tolerance and avoidance mechanisms*

Drought tolerance involves the maintenance of plant metabolism by stomatal control or by induction of adequate cell turgor due to osmoregulators (Touchette *et al.*, 2007). A tolerance mechanism was observed in **Balsam**; this clone maintained greater stomatal aperture, as well as similar $g_s$ rates compared with control plants despite severe drought stress; these plants also showed less vulnerability to xylem cavitation (Table 3-1). Tolerant plants are also able to increase or decrease cell wall rigidity under drought conditions. Both responses are considered advantageous for plants (Schulte, 1992; Saito & Terashima, 2004). The low cell wall elasticity capacity observed in **Northwest**, maintaining lower $\Psi_{leaf}$ without substantial decreases in tissue content (Fig. 3-3, Table 4-6), in addition to maintenance of greater stomatal aperture and lower vulnerability to xylem cavitation positioned this clone as tolerant to drought stress (Figs. 3-4, 3-6). Northwest was also previously characterized as moderately tolerant to drought (Tschaplinski & Blake, 1989; Silim, 2009). **Walker** clone also falls in this category, by decreasing $g_s$ and maintaining $\Psi_{leaf}$ rates comparable to well watered plants, in addition to having moderate vulnerability to xylem cavitation (Fig. 3-2G, Table 3-1). This clone also showed osmotic adjustment by increasing solute concentration in leaves and roots, as a mechanism aimed at maintaining water uptake and cell protection (Table 4-6), and higher $L_{pr}$ uder drought conditions suggesting a better water transport capacity of this clone (Fig.5-2). A previous study which examined field drought response of poplars positioned Walker as moderately tolerant to drought (Silim, 2009).

Drought avoidance mechanism throught the control of water loss or enhanced root water uptake and reduction of $g_s$ as $\Psi_{soil}$ decrease, was also found in this study. **P38P38** clone showed a reduction in stomatal aperture in addition to a decrease in $g_s$ under severe drought (Table 3-1). This clone showed a moderate resistance to xylem vulnerability to induced drought, as well a greater cell wall elasticity maintaining positive turgor to lower $\Psi_{soil}$ (Figs. 3-6 and Table 4-6). In addition, high leaf starch content of this clone during severe drought could play an important role by sustaining cell metabolism when photosynthate production was insufficient to meet the demand. Root hydraulic conductivity values were similar to the controls (Fig 5-2), maintaining water movement from

roots to leaves. Similar avoidance characteristics were observed in **Okanese,** decreasing stomatal aperture and stomatal conductance under severe drought. This clone also decreased $\Psi_{leaf}$ and showed more vulnerability to xylem cavitation (Table 3-1). The relatively low $\varepsilon_{max}$ value (Table 4-6) suggest that this clone has flexible cell walls decreasing the probability of plasmolysis during water loss (Touchette, 2007). Okanese had shown high drought tolerance under field conditions (Silim, 2009).

Mortality of avoidant plants might be induced at prolonged drought periods of intermediate intensity, since these plants are less resistant to xylem cavitation and decreases in stomatal conductance could lead to a "carbon starvation" as described by McDowell *et al.*, 2008.

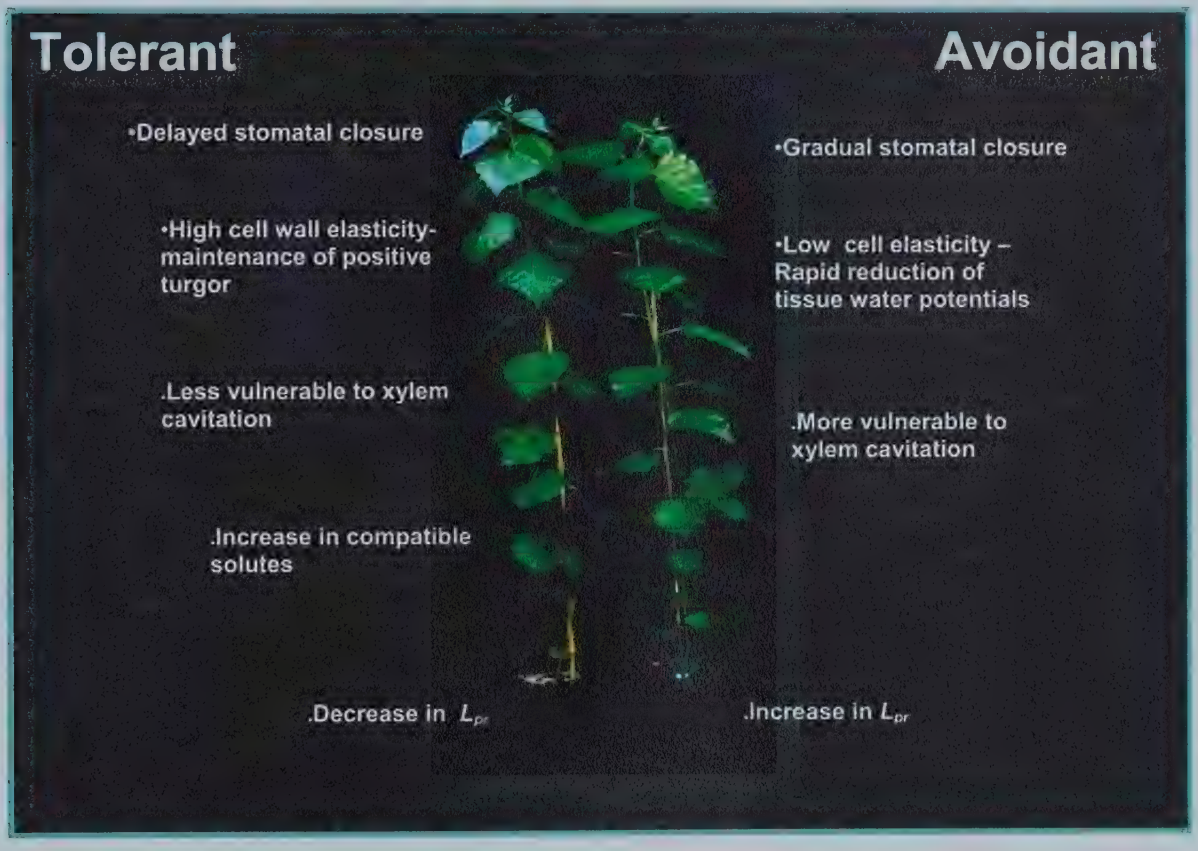

Tolerant
Avoidant
•Delayed stomatal closure
•Gradual stomatal closure
•High cell wall elasticity- maintenance of positive turgor
•Low cell elasticity – Rapid reduction of tissue water potentials
.Less vulnerable to xylem cavitation
.More vulnerable to xylem cavitation
.Increase in compatible solutes
.Decrease in $L_{pr}$
.Increase in $L_{pr}$

**Figure 6-1.** Tolerance and avoidance mechanisms in the studied poplars.

### *6.4 Drought resistance screening*

Overall the results of this research have advanced the understanding of the whole plant response system under drought and recovery conditions. In addition, responses of poplars to different levels of drought are largely clonal. A

functional coordination was found in each poplar under different water regimes. While stomatal regulation and xylem vulnerability to cavitation were related, adjustments in root hydraulic conductivity were probably related to root characteristics and/or solute accumulation capacity. Also osmoregulation, due to carbohydrate and amino acids accumulation, was again clone specific, and dependent on the tissue and water regime in the plant. Fast recovery mechanisms were also observed associated with modifications in root hydraulic conductivity.

Based on my findings and the techniques used to approach drought-resistance mechanisms, I can conclude that:

(i) Measurement of resistance to embolism using the Cavitron technique provided an effective tool for predicting the xylem tension at which drought-embolism will occur in these poplars. This technique also provided useful information for selecting poplar clones suitable for drought conditions.

(ii) The pressure volume technique provided a useful tool to characterized cell water relations of poplars, and also contributed to the understanding of all three mechanisms (osmotic adjustment, leaf cell elasticity and symplastic water content) in the poplar clones.

(iii) The low pressure flow meter technique used to measure cavitation and vessel refilling provided useful information to approach the vessel embolism and refilling mechanism, measured as a percentage of loss of conductivity, and also to differentiate poplars with a greater refilling capacity.

(iv) Measurements of solutes (carbohydrates and total free amino acids) can provide additional information concerning possible involvement of these potential osmolytes in drought resistance and recovery.

(v) Root hydraulic conductivity measurements were helpful to understand root adjustment and response under different drought levels and drought recovery.

(vi) In conclusion, the Cavitron and the pressure volume techniques appear to be useful screening techniques for

identifying poplars with drought-resistance characteristics without previous plant exposure to drought conditions in the field. While tools like the low pressure flow meter technique, measurements of root hydraulic conductivity and accumulation of osmoregulators may be of more practical use when screening plants that are already exposed to drought. According to the Cavitron technique, plants displaying $P_{50}$ values around -1.4 MP would be less vulnerable to cavitation.

### *6.5 Future research*

This research contributed to the understanding of the combined drought-resistance mechanisms in poplar trees, and the recovery capacity after severe drought. However, there are several aspects of drought resistance and recovery that merit further research to better understand these mechanisms and that would enable better interpretation of research results such as those obtained from this study:

(i) Accumulation of proline and total free amino acids were obtained in this study after re-watering, however no indications of organic solutes were observed as a drought resistance mechanism. Thus, more studies of different solutes (e.g. inorganic) are needed in order to understand how osmotic adjustment is possible in leaves.

(ii) Based on the involvement of aquaporins (Kaldenhoff *et al.*, 2008) and solutes (Salleo *et al.*, 2006) in refilling mechanisms in vessels, further studies involving changes in aquaporin expression in cell walls, and accumulation of solutes in phloem will be important to understand in more detail the fast recovery (30 minutes after rewatering) of these poplars after drought treatment.

(iii) Further studies about the morphology and physiology of roots will provide important complementary information of how these trees are able to maintain root hydraulic conductivity under drought stress.

(iv) Finally, it would be important to perform further field studies to compare the results with those found in this research, and understand how different environmental conditions could influence

drought resistance characteristics and how different ecophysiological traits are combined in the field to provide specific adaptations to drought conditions.

## *References*


Bond BJ, Kavanagh KL (1999) Stomatal behaviour of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential. *Tree Physiol.* **19**, 503-510.

Cochard H, Coll Ll, Roux XL, Améglio R (2002) Unravelling the effects of plant hydraulics on stomatal closure during water stress in Walnut. *Plant Physiol.* **128**, 282-290.

Cochard H, Casella E, Mencuccini M (2007) Xylem vulnerability to cavitation varies among poplar and willow clones and correlates with yield. *Tree Physiol.* **27**, 1761-1767.

DesRochers A, van den Driessche R, Thomas BR (2007) The interaction between nitrogen source, soil pH, and drought in the growth and physiology of three poplar clones. *Can J Bot.* **85**, 1046-1057.

Ennajeh M, Tounekti T, Vadel AM, Khemira H, Cochard H (2008) Water relations and drought-induced in olive (*Olea europea*) varieties 'Meski' and "Chemlali" during severe drought. *Tree Physiol.* **28**, 971-976.

Holbrook NM, Zwieniecki MA (1999) Embolism repair and xylem tension: Do we need a miracle? *Plant Physiol.* **120**, 7-10.

Kaldenhoff R, Ribas-Carbo M, Sans JF, Lovisolo C, Heckwolf M, Uehlein N (2008) Aquaporins and plant water balance. *Plant Cell Environ..* **31**, 658-666.

Linton MJ, Sperry JS, Williams DG (1998) Limits to water transport in *Juniperus osteosperma* and *Pinus edulis*: implications for drought tolerance and regulation of transpiration. *Funct Ecol.* **12**, 906-911.

McDowell, N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mostality during drought: why do some plants survive while others succumb to drought? New *Phytol.* **178**,719-739.

Nilsen ET, Orcutt DM (1996) Water dynamics in plants. *In*: Physiology of plants under stress. Abiotic factors. John Wiley and Sons (Eds). Pp. 278-321.

Pearce DW, Millard S, Bray DF, Rood SB (2006) Stomatal characteristics of riparian poplar species in a semi-arid environment. *Tree Physiol.* **26**, 211-218.

Rood SB, Patiño S, Coombs K, Tyree MT (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees.* **14**, 248-257.

Saito T, Terashima I (2004) Reversible decreases in the bulk elastic modulus of mature leaves of deciduous *Quercus* species subjected to two drought treatments. *Plant Cell Environ.* 27,863–75.

Sakr S, Georges A, Morillon R, Maurel K, Decourteix M, Agnes G, Fleurat-Lessard P, Julien JL, Chrispeels MA (2003) Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiol.* **133**, 630-641.

Salleo S, Lo Gullo MA, Trifilò P, Nardini A (2004) New evidence for a role of vessel-associated cells and phloem in the rapid xylem refilling of cavitated stems of *Laurus nobilis* L. *Plant Cell Environ.* **27**, 1065–1076.

Salleo S, Trifilò P, Lo Gullo MA (2006) Phloem as a possible major determinant of rapid cavitation reversal in stems of *Laurus nobilis* (laurel). *Funct Plant Biol.* **33**, 1063-1074.

Schobert B, Tschesche H (1978) Unusual solution properties of proline and its interactions with proteins. *Biochem Biophys Acta* **541**, 240–277.

Schulte PJ (1992) The units of currency for plant water status. *Plant Cell Environ.* **15**, 7:10.

Silim S, Nash R, Reynard D, White B, Shroeder W (2009) Leaf gas exchange and water potential responses to drought in nine poplar (*Populus* spp.) clones with contrasting drought tolerance. *Trees* **23**, 959-969.

Sparks JP, Black RA (1999) Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. *Tree Physiol.* **19**, 453-459.

Spence RD, Wu H, Sharpe PJH, Clark KG (1986) Water stress effects on guard cell anatomy and the mechanical advantage of the epidermal cells. *Plant Cell Environ.* **9**, 197–202.

Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *J Exp Bot* **44**(263), 1075-1082.

Sperry JS, Adler FR, Campbell GS, Comstock JP (1998) Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant Cell Environ.* **21**, 347-359.

Steudle E (2002) Transport of water in plants. *Environ Control Biol.* **40**, 29-37.

Taleisnik E, Peyrano G, Cordoba A, Arias C (1999) Water retention capacity in root segments differing in the degree of exodermis development. *Ann Bot.* **83**, 19–27.

Touchette BW (2007) Seagrass–salinity interactions: physiological mechanisms used by submersed marine angiosperms for a life at sea. *J Exp Mar Biol Ecol* **350**, 194–215.

Touchette BW, Iannacone LR, Turner GE, Frank AR, (2007) Drought tolerance versus drought avoidance: a comparison of plant–water relations in herbaceous wetland plants subjected to water withdrawal and repletion. *Wetlands.* **27**, 656–667.

Tschaplinski TJ, Blake TJ (1989) Water stress tolerance and late-season organic solute accumulation in hybrid poplar. *Can J Bot* .**67**, 1681-1688.

Tschaplinski TJ, Tuskan GA, Gebre GM, Todd DE (1998) Drought resistance of two hybrid *Populus* clones grown in a large-scale plantation. *Tree Physiol.* **18**, 653-658.

Turner NC, Jones MM (1980) Turgor maintenance by osmotic adjustment: A review and evaluation. In: Adaptation of plants to water and high temperature stress. Turner NC, Kramer PJ (Eds). John Wiley & Sons. United States. Pp. 87-103.

Vandeleur R, Niemietz C, Tilbrook J, Tyerman SD (2005) Role of aquaporins in root responses to irrigation. *Plant Soil* **274**, 141-161.

Versules PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant J.* **45**, 523-539.

Wang Y, Zwiazek JJ (1999) Spring changes in water relations, gas exchange, and carbohydrates of white spruce (*Picea glauca*) seedlings. *Can J For Res***29**, 332-338.

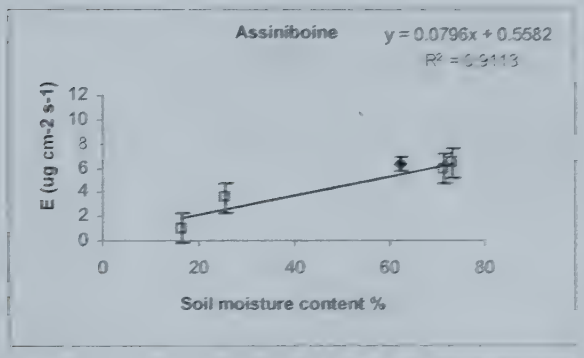
Yin C, Peng Y, Zang R, Zhu Y, Li C (2005) Adaptive responses of *Populus kangdingensis* to drought stress. *Physiol Plant* **123**, 445-451.

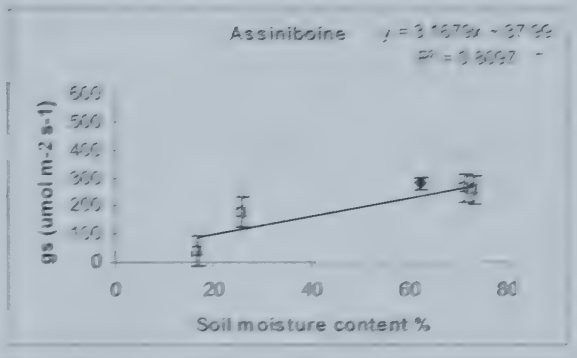
# Appendixes

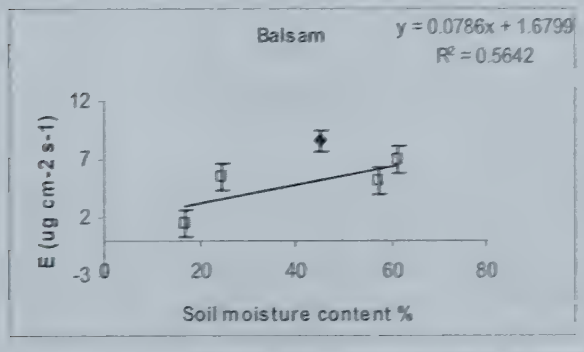
## Appendix A

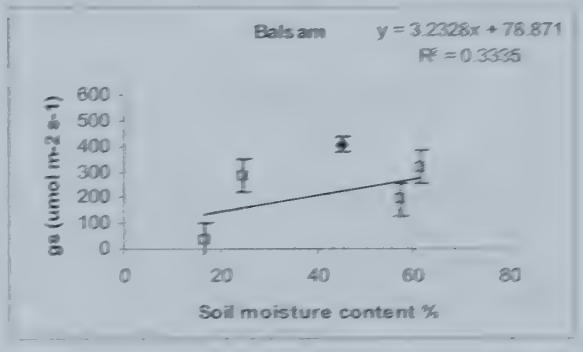
### Preliminary drought experiment

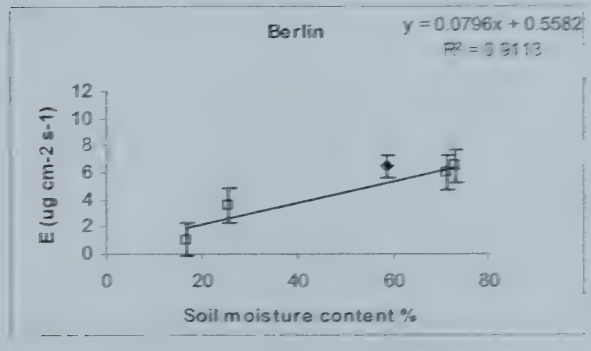
This experiment was conducted in the greenhouse under semi-controlled conditions (as described in numeral 3.2) during summer of 2005. Transpiration and stomatal conductance were measured at different soil water contents to determine the different drought levels for the poplar clones (Figure A-1). Soil water content was decrease until the plants showed wilted leaves.

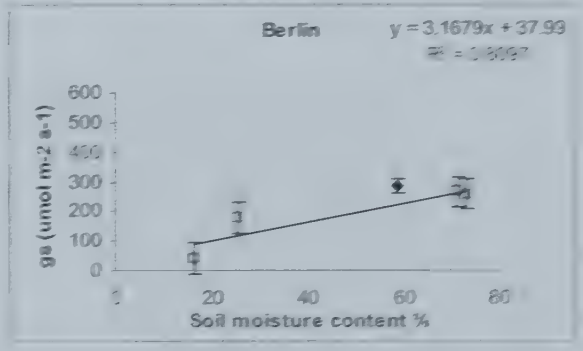


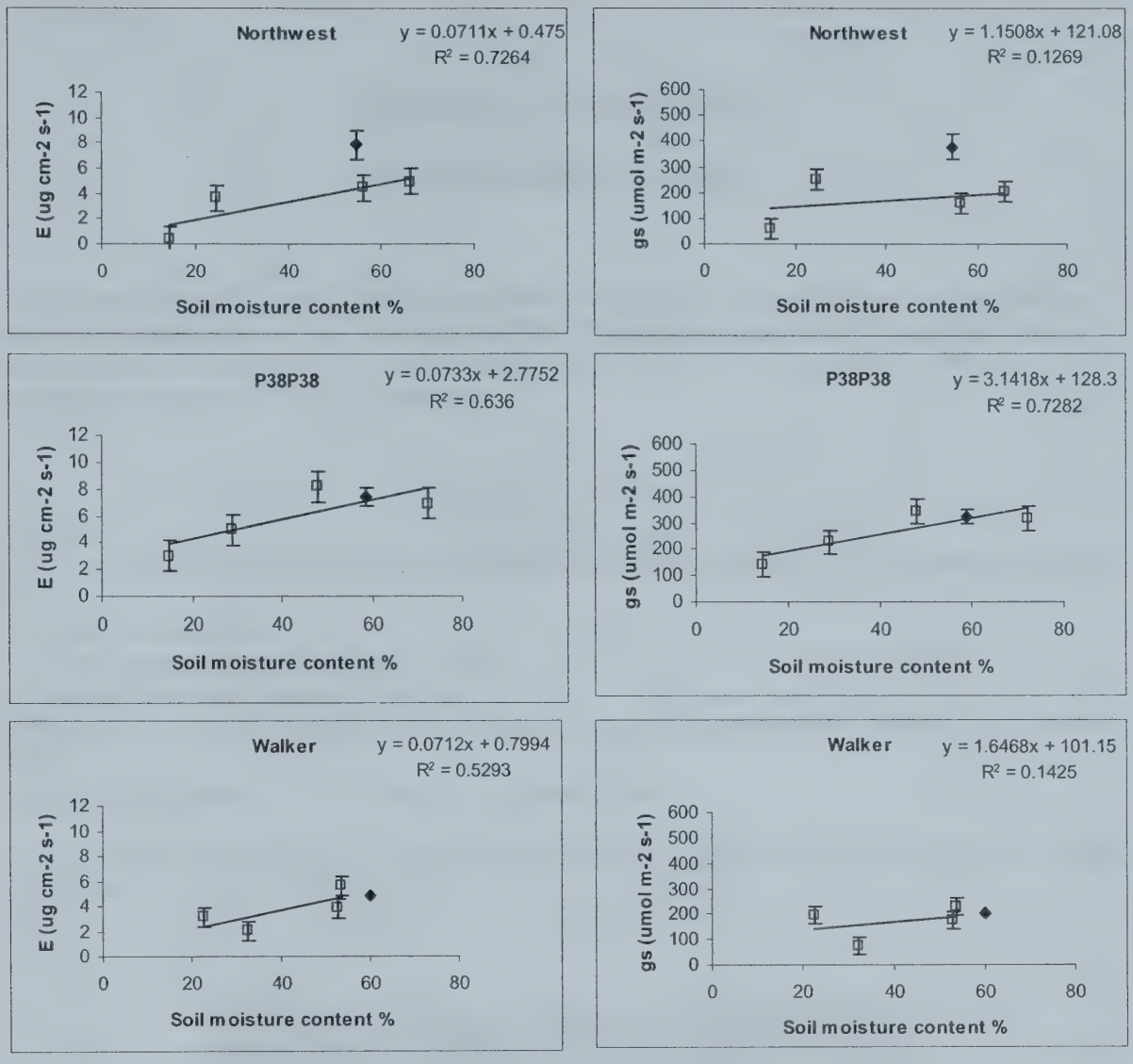



**Figure A-1**. Relationship between transpiration (E) or stomatal conductance ($g_s$) and soil moisture content for poplar clones. Means ± SE for diurnal measurement between 1000 and 1400 h. Different water levels (□) starting from severe (lower soil moisture content value), mild, and two recoveries (R1 and R2) (after 1 day and two days of rewatering after severe drought) are correlated. Control plants (♦) were used as reference.

# Apendix B. Chapter three.

## Statistical data analysis

**1. Statistical models and ANOVA tables for soil water potential, gas exchange and leaf water potentials (Figs. 3.1, 3.2 and 3.3).** Degrees of freedom of numerator and denominator, F-values and p-values are shown. Effects were tested for significance at alpha=0.05 using proc mixed (SAS 9.1)

$$Y_{ijk} = \mu + B_i + T_j + (BT)_{ij} + \varepsilon_{ijk}$$

where

$Y_{ijk}$: is the soil water potential ($\Psi_{leaf}$) stomatal conductance ($g_s$), or leaf water potential ($\Psi_{leaf}$),

$\mu$: is the overall mean

$B_i$: is the random effect of $i^{th}$ block (i=1-2)

$T_j$: is the $j^{th}$ effect of treatment (j=1-5)

$(BT)_{ij}$: is the random interaction effect of the $i^{th}$ level of block with the $j^{th}$ level of treatment, and

$\varepsilon_{ijk}$: is the error term (k= 1,2...number of observations)

The treatments were considered as fixed factors. Block and its interactions were considered as random factors in the analysis.

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **block** | 2 | Block. 1 Block. 2 |
| **treat** | 5 | Control Mild Severe Rec.30 Rec. 2 days |

### 1.1. Assiniboine

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | **Variable** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatment** | $\Psi_{soil}$ | 4 | 40 | 118.11 | <.0001 |
| **Treatment** | $g_s$ | 4 | 26 | 13.29 | <.0001 |
| **Treatment** | $\Psi_{leaf}$ | 4 | 25 | 4.03 | 0.0117 |

### 1.2 P38P38

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | $\Psi_{soil}$ | 4 | 28 | 113.17 | <.0001 |
| Treatment | $g_s$ | 4 | 29 | 8.85 | <.0001 |
| Treatment | $\Psi_{leaf}$ | 4 | 29 | 6.43 | 0.0008 |

### 1.3 Northwest

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | $\Psi_{soil}$ | 4 | 40 | 148.86 | <.0001 |
| Treatment | $g_s$ | 4 | 25.1 | 2.92 | 0.0414 |
| Treatment | $\Psi_{leaf}$ | 4 | 24 | 5.59 | 0.0025 |

### 1.4 Okanese

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | $\Psi_{soil}$ | 4 | 39 | 145.96 | <.0001 |
| Treatment | $g_s$ | 4 | 26 | 4.35 | 0.0079 |
| Treatment | $\Psi_{leaf}$ | 4 | 26 | 7.27 | 0.0005 |

### 1.5 Berlin

**Note: There were four treatments (Control, Mild, Severe and recovery after 2 days) analyzed for this clone.**

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | $\Psi_{soil}$ | 3 | 11 | 104.66 | <.0001 |
| Treatment | $g_s$ | 3 | 11 | 3.27 | 0.0630 |
| Treatment | $\Psi_{leaf}$ | 3 | 11 | 14.32 | 0.0004 |

### 1.6 Balsam

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | $\Psi_{soil}$ | 4 | 38 | 179.42 | <.0001 |
| Treatment | $g_s$ | 4 | 24.1 | 3.13 | 0.0332 |
| Treatment | $\Psi_{leaf}$ | 4 | 25 | 12.30 | <.0001 |

### 1.7 Walker

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | $\Psi_{soil}$ | 4 | 32.2 | 74.91 | <.0001 |
| Treatment | $g_s$ | 4 | 23 | 2.91 | 0.0439 |
| Treatment | $\Psi_{leaf}$ | 4 | 22.1 | 4.59 | 0.0075 |

**2. Statistical model and ANOVA tables for stomata aperture (Fig. 3.4).** Degrees of freedom of numerator and denominator, F-values and p-values are shown. Effects were tested for significance at alpha=0.05 using proc mixed (SAS 9.1)

The model for the completely randomized design for the stomata aperture was;

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where
$Y_{ij}$: is the stomata aperture
$\mu$: is the overall mean
$T_i$: is $i^{th}$ effect of treatment (i=1-2), and
$\varepsilon_{ij}$: is the error term (j= 1,2...number of observations)

Treatments were considered fixed factors in the analysis.

### 2.1. Assiniboine

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Treatment | 1 | 223 | 10.09 | 0.001 |

2.2. P38P38

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Treatment | 1 | 89 | 13.74 | 0.0004 |

2.3. Northwest

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Treatment | 1 | 196 | 1.23 | 0.27 |

2.4. Okanese

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Treatment | 1 | 216 | 83.16 | <0.0001 |

2.5. Berlin

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Treatment | 1 | 262 | 125.57 | <0.0001 |

2.6. Balsam

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Treatment | 1 | 216 | 83.16 | <0.0001 |

2.7. Walker

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Treatment | 1 | 216 | 83.16 | <0.0001 |

**3. Statistical model and ANOVA tables for leaf stomatal density and size (Fig. 3.5).** Degrees of freedom of numerator and denominator, F-values and p-values are shown. Effects were tested for significance at alpha=0.05 using proc mixed (SAS 9.1)

$$Y_{ij} = \mu + C_i + \varepsilon_{ij}$$

where

$Y_{ij}$: is the stomata density or size

$\mu$: is the overall mean

$C_i$: is $i^{th}$ effect of clone (i=1-7), and

$\varepsilon_{ij}$: is the error term (j= 1,2...number of observations)

Clones were considered fixed factors in the analysis.

### 3.1 Stomata density

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Clone | 6 | 34 | 11.77 | <0.0001 |

### 3.2 Stomata size

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Clone | 6 | 33 | 65.36 | <0.0001 |

**4. Generalized nonlinear regression model for xylem vulnerability curves (Fig. 3.6)**

Model using fixed effects for all poplar clones and random effects for individual samples is shown. Effects were tested for significance at alpha=0.01 using mixed model with nlme.

*Model*:

PLC = SSlogis2p(Tension, xmid, scal)

Where:

SSlogis = is the Asym/2 at xmid

xmid = determines the position of the curve on the abscissa and gives the water potential corresponding to 50% loss of conductivity

scal = slope of the curve

| Fixed effects | Value | Std. Error | DF | t-value | P-value |
|---|---|---|---|---|---|
| xmid | -1.3099663 | 0.03053566 | 511 | -42.89956 | <0.0001 |
| scal | -0.1608754 | 0.01245254 | 511 | -12.91908 | <0.0001 |

**5. Statistical model and ANOVA tables for loss of conductivity at 10, 50 and 90% ($P1_0$ $P_{50}$ $P9_0$) (Fig. 3.7).** Degrees of freedom of numerator and denominator, F-values and p-values are shown. Effects were tested for significance at alpha=0.05 using proc mixed (SAS 9.1)

$$Y_{ij} = \mu + C_i + \varepsilon_{ij}$$

where

$Y_{ij}$: is the loss of conductivity at 10, 50 and 90% ($P_{10}$, $P_{50}$ and $P_{90}$)

$\mu$: is the overall mean

$C_i$: is the $i^{th}$ effect of clone (i=1-7), and

$\varepsilon_{ij}$: is the error term (j= 1,2...number of observations)

Clones were considered as fixed factors in the analysis.

### 5.1 Loss of conductivity at 10% ($P_{10}$)

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| clone | 5 | 30 | 27.37 | <.0001 |

### 5.2 Loss of conductivity at 50% ($P_{50}$)

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| clone | 5 | 30 | 32.41 | <.0001 |

### 5.3 Loss of conductivity at 90% ($P_{90}$)

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| clone | 5 | 30 | 14.32 | <.0001 |

## Appendix C. Chapter three.

### *Retention curve for soil water potential*

Soil volumetric water content at potentials of $-1.0 \cdot 10^{-4}$, $-3.0 \cdot 10^{-4}$, $-8.0 \cdot 10^{-4}$, $-1.7 \cdot 10^{-3}$, $-2.6 \cdot 10^{-3}$, $-3.5 \cdot 10^{-3}$ and $-6.5 \cdot 10^{-3}$ MPa were determined using the tension table method (Figure C-1) (Reynolds and Topp, 2008). A 15 cm diameter tension table was constructed using 1.2 mm nylon filter membrane (GE Magna Nylon membrane) with a bubbling pressure of $-1 \cdot 10^{-2}$ MPa. Metro Mix® 290 was packed inside a 15 cm high column on top of the tension table to a dry bulk density of 0.2 g $cm^{-3}$, and horizontal TDR probes were inserted into the soil at 5, 7, 9, 10 and 11 cm above the tension table. The filter membrane allowed the Metro Mix ® to stay in hydraulic contact with a water reservoir even under negative pressure. Water potential is then determined by measuring the distance between the TDR probes and the water level in the reservoir. The Metro Mix® 290 was initially saturated and allowed to drain by lowering the water reservoir below the tension table. Soil water content was monitored with the TDR probes. Water content usually stabilized after three to five days of drainage and it was assumed to be at the potential calculated with the distance between the water level in the reservoir and the TDR probe.

Soil volumetric water content at potentials of $-2.0 \cdot 10^{-2}$, $-2.5 \cdot 10^{-2}$, -0.18, -0.31, and -5.5 MPa were measured with the freezing point depression technique (Figure C-1) (Suzuki, 2004). Metro Mix® was packed into 5 - 20 ml syringes to achieve a dry bulk density of 0.2 g $cm^{-3}$. Distilled water was then added to achieve volumetric water contents of 30, 25, 20, 15, and 10%. Copper-constantin thermocouples were inserted in each syring such that they were in the middle of the soil sample. The soil samples were placed in a freezer and soil temperature was monitored with a Campbell Scientific 21X data logger. The freezing point depression of pure water was also measured with the thermocouples to determine appropriate temperature offsets. Suzuki, 2004 gives the following relationship between soil water potential and freezing point depression:

$$\Psi = 1.23 \cdot T - \pi \qquad [1]$$

where T is the freezing point depression, and $\pi$ is the osmotic potential. Since distilled water was used the osmotic potential was assumed to be negligible. Equation [1] was derived using the Clapeyron equation from thermodynamics.

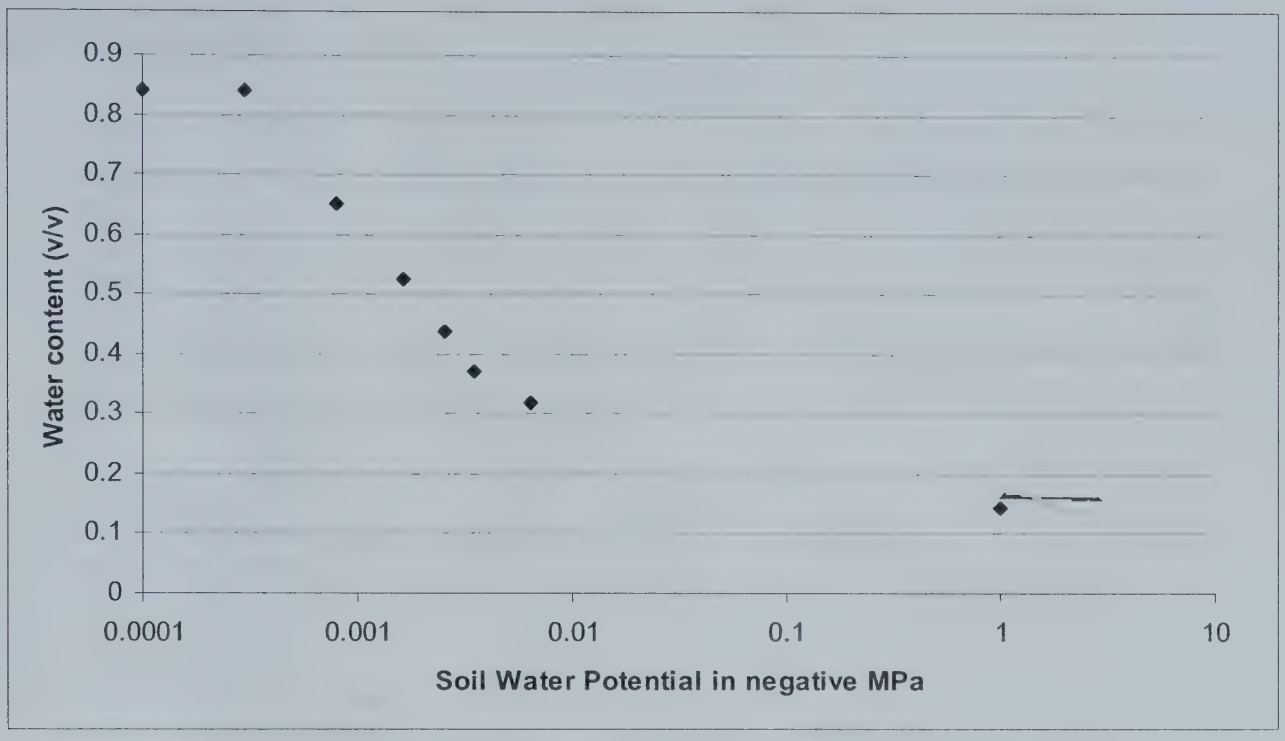



**Figure C-1.** Retention curve for Metro Mix ® soil. Wetter points of the curve (♦) were obtained with the pressure plate and tension table method. Drier points (—) were then determined using the freezing point depression method.

The curve was fitted (Figure C-2) using a Groenevelt/Cameron model as follows:

$gr(\Psi, \theta s, \theta w, d, k0, n): = \theta s - (\theta s - \theta w) \exp [(k0/d)^n - (k0/\Psi)^n]$

Where $\Psi$ is potential, $\theta s$ is the saturated water content, k0 and n are fitting parameters, d is the potential where water content is 0=10E+4.9 meters.

Defining $\theta$ and $\theta w$ here constrains the curve between the first and last data points.

$\theta$ = 0.841 (saturated water content obtained for Metro Mix® 290)
$\theta w$= 0.1435
d= 100 (this is the potential where water content=0)

| Ψ | gr(Ψ,θ, θw, d, k0, n) |
|---|---|
| 0.01 | 0.840721 |
| 0.02 | 0.831606 |
| 0.03 | 0.809586 |
| 0.04 | 0.7812 |
| 0.05 | 0.751285 |
| 0.06 | 0.722154 |
| 0.07 | 0.694751 |
| 0.08 | 0.669376 |
| 0.09 | 0.646037 |
| 0.1 | 0.62462 |
| 0.11 | 0.604965 |
| 0.12 | 0.586901 |
| 0.13 | 0.570266 |
| 0.14 | 0.554909 |
| 0.15 | 0.540698 |
| 0.16 | 0.527512 |

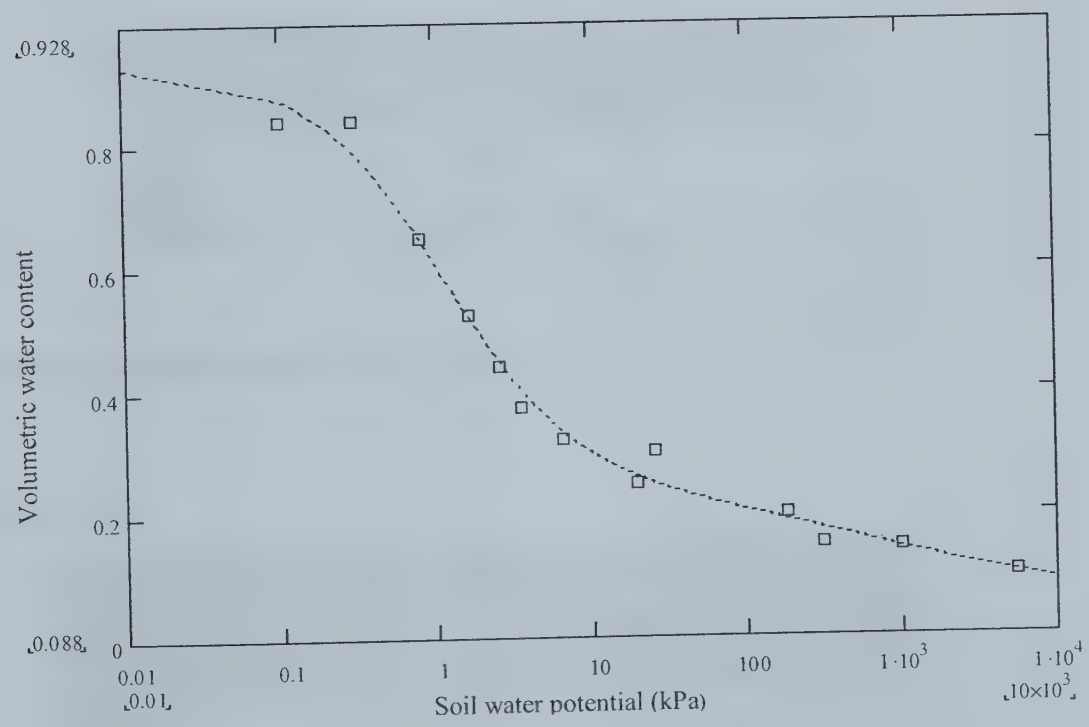



**Figure C-2.** Retention fitted curve using Groenevelt /Cameron model. Measured values (□) and fitted model (- - ) are shown.

## Appendix D. Chapter four

### Statistical data analysis

**1. Statistical models and ANOVA tables for non-structural carbohydrates (Table 4.1, 4.2) (Fig. 4.2)** Degrees of freedom of numerator and denominator, F-values and p-values are shown. Effects were tested for significance at alpha=0.05 using proc mixed (SAS 9.1)

$$Y_{ijk} = \mu + B_i + T_j + (BT)_{ij} + \varepsilon_{ijk}$$

where
$Y_{ijk}$: is the starch in leaves or sugar concentration in leaves and roots,
$\mu$: is the overall mean
$B_i$: is the random effect of $i^{th}$ block (i=1-2)
$T_j$: is the $j^{th}$ effect of treatment (j=1-5)
$(BT)_{ij}$: is the random interaction effect of the $i^{th}$ level of block with the $j^{th}$ level of treatment, and
$\varepsilon_{ijk}$: is the error term (k= 1,2...number of observations)

The treatments were considered as fixed factors. Block and its interactions were considered as random factors in the analysis.

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **block** | 2 | Block. 1 Block. 2 |
| **treat** | 5 | Control *vs* water level |

**Sugar concentration in poplar leaves (Table 4.1)**

**1.1 Assiniboine**

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 9 | 0.06 | 0.8072 |
| **Treat** | Severe *vs* Control | 1 | 11 | 1.77 | 0.2104 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 6 | 0.00 | 0.9801 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 13 | 0.82 | 0.3810 |

### 1.2 P38P38

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 4 | 3.52 | 0.1339 |
| Treat | Severe *vs* Control | 1 | 6 | 0.14 | 0.7168 |
| Treat | Rec. 30 min. *vs* Control | 1 | 5 | 0.62 | 0.4662 |
| Treat | Rec. 2 days *vs* Control | 1 | 7 | 2.63 | 0.1490 |

### 1.3 Northwest

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 9 | 14.26 | 0.0044 |
| Treat | Severe *vs* Control | 1 | 13 | 1.01 | 0.3341 |
| Treat | Rec. 30 min. *vs* Control | 1 | 9 | 1.78 | 0.2145 |
| Treat | Rec. 2 days *vs* Control | 1 | 9.11 | 0.02 | 0.8802 |

### 1.4 Okanese

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 13 | 0.46 | 0.5083 |
| Treat | Severe *vs* Control | 1 | 13 | 0.01 | 0.9074 |
| Treat | Rec. 30 min. *vs* Control | 1 | 10 | 1.98 | 0.1899 |
| Treat | Rec. 2 days *vs* Control | 1 | 15 | 0.95 | 0.3444 |

### 1.5 Berlin

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 5 | 0.27 | 0.6283 |
| Treat | Severe *vs* Control | 1 | 5 | 0.45 | 0.5323 |
| Treat | Rec. 2 days *vs* Control | 1 | 8 | 0.00 | 0.9752 |

### 1.6 Balsam

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 11.2 | 0.11 | 0.7479 |
| Treat | Severe *vs* Control | 1 | 8 | 2.21 | 0.1754 |
| Treat | Rec. 30 min. *vs* Control | 1 | 5 | 0.67 | 0.4505 |
| Treat | Rec. 2 days *vs* Control | 1 | 10 | 2.88 | 0.1207 |

### 1.7 Walker

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 10 | 0.57 | 0.4678 |
| Treat | Severe *vs* Control | 1 | 8.04 | 0.39 | 0.5494 |
| Treat | Rec. 30 min. *vs* Control | 1 | 14 | 0.12 | 0.7386 |
| Treat | Rec. 2 days *vs* Control | 1 | 10 | 0.43 | 0.5264 |

## Sugar concentration in poplar roots (Table 4.2)

### 1.1 Assiniboine

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 4 | 0.19 | 0.6843 |
| Treat | Severe *vs* Control | 1 | 3 | 0.03 | 0.8703 |
| Treat | Rec. 30 min. *vs* Control | 1 | 5 | 0.89 | 0.3898 |
| Treat | Rec. 2 days *vs* Control | 1 | 5 | 1.96 | 0.2208 |

### 1.2 P38P38

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 10 | 0.33 | 0.5780 |
| **Treat** | Severe *vs* Control | 1 | 8 | 0.33 | 0.5833 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 9 | 3.10 | 0.1122 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 10 | 0.24 | 0.6369 |

### 1.3 Northwest

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 3 | 5.32 | 0.1043 |
| **Treat** | Severe *vs* Control | 1 | 3 | 0.00 | 0.9511 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 5 | 0.08 | 0.7873 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 3 | 22.90 | 0.0173 |

### 1.4 Okanese

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 3 | 0.12 | 0.7545 |
| **Treat** | Severe *vs* Control | 1 | 2.01 | 165.77 | 0.0059 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 5.57 | 0.27 | 0.6262 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 4 | 7.43 | 0.0527 |

### 1.5 Walker

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 4 | 0.02 | 0.8933 |
| Treat | Severe *vs* Control | 1 | 4 | 2.90 | 0.1636 |
| Treat | Rec. 30 min. *vs* Control | 1 | 5 | 1.73 | 0.2451 |
| Treat | Rec. 2 days *vs* Control | 1 | 3 | 4.02 | 0.1385 |

## Starch concentration in poplar leaves (Fig. 4.2)

### 1.1 Assiniboine

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 8 | 1.65 | 0.2353 |
| Treat | Severe *vs* Control | 1 | 2.34 | 0.31 | 0.6242 |
| Treat | Rec. 30 min. *vs* Control | 1 | 5 | 2.82 | 0.1542 |
| Treat | Rec. 2 days *vs* Control | 1 | 8.07 | 0.04 | 0.8429 |

### 1.2 P38P38

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 4 | 55.86 | 0.0017 |
| Treat | Severe *vs* Control | 1 | 6 | 11.32 | 0.0151 |
| Treat | Rec. 30 min. *vs* Control | 1 | 7 | 2.54 | 0.1551 |

### 1.3 Northwest

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 1 | 0.75 | 0.5452 |
| **Treat** | Severe *vs* Control | 1 | 7.09 | 0.30 | 0.5981 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 8 | 1.06 | 0.3341 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 6.09 | 0.02 | 0.8902 |

### 1.4 Okanese

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 6 | 0.00 | 0.9935 |
| **Treat** | Severe *vs* Control | 1 | 6 | 1.97 | 0.2095 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 10 | 0.98 | 0.3463 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 8.07 | 0.04 | 0.8429 |

### 1.5 Balsam

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 2.63 | 0.06 | 0.8308 |
| **Treat** | Severe *vs* Control | 1 | 2.81 | 0.00 | 0.9586 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 5 | 1.30 | 0.3066 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 2.32 | 0.04 | 0.8632 |

**1.6 Walker**

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 10 | 0.04 | 0.8435 |
| **Treat** | Severe *vs* Control | 1 | 6.05 | 0.28 | 0.6135 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 2 | 0.61 | 0.5156 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 1 | 0.42 | 0.6330 |

**2. Statistical models and ANOVA tables for total amino acids and proline in leaves and roots (Tables 4-3, 4-4) (Figs. 4-3, 4-4).** Degrees of freedom of numerator and denominator, F-values and p-values are shown. Effects were tested for significance at alpha=0.05 using proc mixed (SAS 9.1)

$$Y_{ijk} = \mu + B_i + T_j + (BT)_{ij} + \varepsilon_{ijk}$$

where
$Y_{ijk}$: is the proline or total amino acid concentration in roots or leaves
$\mu$: is the overall mean
$B_i$: is the random effect of $i^{th}$ block (i=1-2)
$T_j$: is the $j^{th}$ effect of treatment (j=1-5)
$(BT)_{ij}$: is the interaction effect of the $i^{th}$ level of block with the $j^{th}$ level of treatment, and
$\varepsilon_{ijk}$: is the error term (k= 1,2...number of observations)

The treatments were considered as fixed factors. Block and its interactions were considered as random factors in the analysis.

**A. Amino acids and poplar concentration in poplar leaves (Tables 4.3, 4.4)**

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **block** | 2 | Block. 1 Block. 2 |
| **treat** | 4 | Control, Mild, Severe, Rec. 2 days |

### A.2.1 Assiniboine

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (leaves) | 3 | 4 | 0.12 | 0.9463 |
| Treatment | Proline (leaves) | 3 | 12 | 0.63 | 0.6095 |

### A.2.2 P38P38

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (leaves) | 3 | 16.1 | 0.43 | 0.7377 |
| Treatment | Proline (leaves) | 3 | 4.27 | 0.35 | 0.7918 |

### A.2.3 Northwest

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (leaves) | 3 | 24.1 | 0.39 | 0.7612 |
| Treatment | Proline (leaves) | 3 | 2.75 | 0.68 | 0.6227 |

### A. 2.4 Berlin

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (leaves) | 3 | 12 | 2.36 | 0.1225 |
| Treatment | Proline (leaves) | 3 | 4.46 | 0.19 | 0.8969 |

### A.2.5 Balsam

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (leaves) | 3 | 3.76 | 0.53 | 0.6887 |
| Treatment | Proline (leaves) | 3 | 2.65 | 1.96 | 0.3143 |

### A.2.6 Walker

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (leaves) | 3 | 8 | 5.19 | 0.0278 |
| Treatment | Proline (leaves) | 3 | 8 | 11.31 | 0.0030 |

## B. Amino acids and poplar concentration in poplar roots (Figs. 4.3, 4.4)

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| block | 2 | Block. 1 Block. 2 |
| treat | 5 | Control, Mild, Severe, Rec. 30 minutes, Rec. 2 days |

### B.2.1 Assiniboine

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (roots) | 4 | 20 | 4.98 | 0.0059 |
| Treatment | Proline (roots) | 4 | 19.2 | 2.12 | 0.1183 |

### B.2.2 P38P38

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (roots) | 4 | 23 | 0.94 | 0.4579 |
| Treatment | Proline (roots) | 4 | 23 | 1.20 | 0.3367 |

### B.2.3 Northwest

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (roots) | 4 | 18 | 4.36 | 0.0122 |
| Treatment | Proline (roots) | 4 | 18 | 2.95 | 0.0489 |

### B.2.4 Okanese

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (roots) | 4 | 3.24 | 3.29 | 0.1662 |
| Treatment | Proline (roots) | 4 | 17.9 | 2.02 | 0.1349 |

### B.2.5 Balsam

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (roots) | 4 | 41.8 | 5.73 | 0.0557 |
| Treatment | Proline (roots) | 4 | 15 | 4.00 | 0.0211 |

### B.2.6 Walker

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | Variable | Num DF | Den DF | F Value | Pr > F |
| Treatment | Amino acids (roots) | 4 | 18 | 6.19 | 0.0026 |
| Treatment | Proline (roots) | 4 | 18 | 8.46 | 0.0005 |

**3. Statistical models and ANOVA tables for the pressure volume assay (Table 4-5).**
Degrees of freedom of numerator and denominator, F-values and p-values are shown. Effects were tested for significance at alpha=0.05 using proc mixed (SAS 9.1)

$$Y_{ij} = \mu + C_j + \varepsilon_{ij}$$

where
$Y_{ij}$: is the elasticity modulus ($\varepsilon_{max}$), osmotic potential at turgor loss point ($\pi_{tlp}$), osmotic potential at full hydration ($\pi_{ft}$), symplastic water fraction ($R^*_s$), or relative water content (1-$RWC_{TLP}$).
$\mu$: is the overall mean
$C_j$: is the $j^{th}$ effect of clone (j=1-7), and

$\varepsilon_{ij}$: is the error term (j= 1,2...number of observations)

The treatments were considered as fixed factors.

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Clone** | 7 | Assiniboine Balsam Berlin NW Okanese P38P38 Walker |

| | Differences of Least Squares Means in poplar clones | | | |
|---|---|---|---|---|
| **Effect Clone** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| $\pi_{ft}$ | 6 | 41 | 3.59 | 0.006 |
| $\pi_{TLP}$ | 6 | 41 | 4.97 | 0.0007 |
| $RWC_{TLP}$ | 6 | 41 | 5.22 | 0.0005 |
| Symplastic fraction | 6 | 41 | 9.75 | <.0001 |
| $\varepsilon_{max}$ | 6 | 41 | 3.45 | 0.007 |

**Appendix E. Chapter five**

## Statistical data analysis

**1. Statistical models and ANOVA tables for transpiration and root hydraulic conductivity (Figs. 5.2, 5.3)** Degrees of freedom of numerator and denominator, F-values and p-values are shown. Effects were tested for significance at alpha=0.05 using proc mixed (SAS 9.1)

$$Y_{ijk} = \mu + B_i + T_j + (BT)_{ij} + \varepsilon_{ijk}$$

where
$Y_{ijk}$: is the starch in leaves or sugar concentration in leaves and roots,
$\mu$: is the overall mean
$B_i$: is the random effect of $i^{th}$ block (i=1-2)
$T_j$: is the $j^{th}$ effect of treatment (j=1-5)
$(BT)_{ij}$: is the random interaction effect of the $i^{th}$ level of block with the $j^{th}$ level of treatment, and
$\varepsilon_{ijk}$: is the error term (k= 1,2...number of observations)

The treatments were considered as fixed factors. Block and its interactions were considered as random factors in the analysis.

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **block** | 2 | Block. 1 Block. 2 |
| **treat** | 5 | Control *vs* water level |

### A. Transpiration rates of poplar clones (Fig. 5.1)

#### A.1.1 Assiniboine

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 13 | 0.60 | 0.4525 |
| **Treat** | Severe *vs* Control | 1 | 7 | 737.55 | <.0001 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 7 | 7.29 | 0.0306 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 11 | 0.00 | 0.9569 |

### A.1.2 P38P38

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 17 | 2.81 | 0.1118 |
| **Treat** | Severe *vs* Control | 1 | 4.02 | 41.96 | 0.0029 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 5 | 1.44 | 0.2833 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 11 | 2.88 | 0.1175 |

### A.1.3 Northwest

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 9 | 8.93 | 0.0152 |
| **Treat** | Severe *vs* Control | 1 | 6.01 | 174.46 | <.0001 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 10 | 64.67 | <.0001 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 5 | 15.83 | 0.0105 |

### A.1.4 Balsam

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 1 | 7.83 | 0.2186 |
| **Treat** | Severe *vs* Control | 1 | 6 | 85.67 | <.0001 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 1 | 175.07 | 0.0480 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 7 | 4.55 | 0.0703 |

### A.1.5 Berlin

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 16 | 10.08 | 0.0059 |
| **Treat** | Severe *vs* Control | 1 | 8.01 | 50.83 | <.0001 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 9 | 2.18 | 0.1740 |

### A.1.6 Walker

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 11 | 20.68 | 0.0008 |
| Treat | Severe *vs* Control | 1 | 6.04 | 10.59 | 0.0172 |
| Treat | Rec. 30 min. *vs* Control | 1 | 1 | 0.47 | 0.6178 |
| Treat | Rec. 2 days *vs* Control | 1 | 8.01 | 2.50 | 0.1526 |

## B. Root hydraulic conductivity of poplar clones (Fig. 5.2)

### B.1.1 Assiniboine

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 2.19 | 1.08 | 0.3987 |
| Treat | Severe *vs* Control | 1 | 8 | 20.22 | 0.0020 |
| Treat | Rec. 30 min. *vs* Control | 1 | 8 | 6.53 | 0.0338 |
| Treat | Rec. 2 days *vs* Control | 1 | 12 | 3.40 | 0.0901 |

### B.1.2 P38P38

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| Effect | | Num DF | Den DF | F Value | Pr > F |
| Treat | Mild *vs* Control | 1 | 18 | 0.42 | 0.5259 |
| Treat | Severe *vs* Control | 1 | 1 | 2.81 | 0.3422 |
| Treat | Rec. 30 min. *vs* Control | 1 | 6 | 1.29 | 0.2993 |
| Treat | Rec. 2 days *vs* Control | 1 | 12 | 6.96 | 0.0216 |

### B.1.3 Northwest

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 10 | 3.45 | 0.0931 |
| **Treat** | Severe *vs* Control | 1 | 9 | 0.06 | 0.8134 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 11 | 0.15 | 0.7057 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 5 | 0.05 | 0.8322 |

### B.1.4 Balsam

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 11 | 3.55 | 0.0864 |
| **Treat** | Severe *vs* Control | 1 | 6 | 2.56 | 0.1610 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 9 | 37.71 | 0.0002 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 8 | 0.11 | 0.7526 |

### B.1.5 Berlin

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 1.9 | 3.29 | 0.2180 |
| **Treat** | Severe *vs* Control | 1 | 1 | 0.13 | 0.7806 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 9 | 2.18 | 0.1740 |

### B.1.6 Walker

| Type 3 Tests of Fixed Effects | | | | | |
|---|---|---|---|---|---|
| **Effect** | | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treat** | Mild *vs* Control | 1 | 12 | 9.02 | 0.0110 |
| **Treat** | Severe *vs* Control | 1 | 1 | 1.31 | 0.4573 |
| **Treat** | Rec. 30 min. *vs* Control | 1 | 1 | 1.31 | 0.4573 |
| **Treat** | Rec. 2 days *vs* Control | 1 | 1.82 | 1.00 | 0.4304 |

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